Food and furniture:

Disentangling trophic and non-trophic interactions within foundation species' communities

Anna Catharina Wilhelmina Borst

Cover photo's show: *Tillandsia usnoides* (Spanish moss)

Front illustration represents Spanish moss as the foundation species as the central species of the trophic network.

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Food and Furniture

Disentangling trophic and non-trophic interactions within foundation species communities

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1. General introduction

Vervang door opgemaakte versie

Eat and be eaten

Charles Darwin, contemplating the theory of evolution, found evidence for the process of natural selection in the limitation of resources. He noticed that organisms often have manifold offspring, even though population levels tend to remain stable over time. Therefore, he concluded that a lack of resources for overabundant individuals resulted in a struggle for life, favoring only those best adapted to the environment, while eliminating others in 'the war of nature'. He found evidence for this theory when he realized that the beaks of otherwise similar finches in the Galapagos were highly adapted to the primary food they consumed (Darwin 1859).

Ever since the evolution of first life, the struggle for existence has begun. Photoautotrophic primary producers such as cyanobacteria, algae and plants create biomass from CO_2, minerals and energy from the sun. In addition, chemoautotrophic primary producers create biomass from CO_2 and nutrients, but gain their energy from the oxidation of chemical compounds. Both groups of autotrophs are considered the first level of the food chain, called the first trophic (feeding-related) level. Herbivores and bacterivores are the first line of consumers which directly consume these primary producers, and are eaten themselves by predators and omnivores, creating a food chain (Elton 1927). Only a fraction of the energy (~10%) that is consumed can be stored by the next trophic level, much energy is lost along the trophic chain either by excretion or respiration (Elton 1927; Lindeman 1942). All of the biomass in this food chain is eventually broken down by detritivores (which are also eaten themselves) and the cycle starts again.

A quest for resources

Resources are defined as substances or objects required by organisms for normal growth and fitness, that can be consumed. If the availability of a resource is limited for more than one organism, this will normally lead to exploitation competition. In general, only really 'consumable' components such as carbon dioxide, photons, nutrients, prey, and water are defined as resources, but in principle also other 'objects' that can be utilized by an organism including space, pollination and protection can be defined as a resource (Begon *et al.* 1996).

Trophic interactions

A trophic interaction is a consumer-resource interaction in which energy and/or nutrient transfer from one organism to another occurs (e.g. predator-prey, plant-herbivore, host-parasite) (Holland & DeAngelis 2010). Every organism is a consumer obtaining resources to grow, maintain and reproduce, and is generally involved in multiple consumer-resource

interactions. For instance, exploitation interactions (e.g. predator-prey, host-parasite) have a facilitation/inhibition (+/-) outcome because exploiter benefits, while the exploited suffers.

In general, exploitation interactions occur between organisms within a trophic context. They are often studied as predator-prey interactions that are classically described as one animal eating another. However, herbivore-plant interactions can be both predator-prey interactions (seed consumption) and parasite-host interactions (leaf consumption) and are therefore also exploitation interactions (Begon *et al.* 1996). Predator-prey, herbivore-plant and parasite-host interactions have in common that the consumer benefits at the expense of the resource organism, a facilitation/inhibition-type (+/-) interaction.

Ever since Darwin, trophic interactions have been seen as the main drivers of evolutionary and ecological patterns in species dynamics and have been the focus of countless studies trying characterize populations and communities of species co-existing over time (Fig. 1). Alfred Lotka, and later Vito Volterra, individually modeled the interaction between predator and prey, hypothesizing that prey densities determine predator densities and vice versa, laying the basis of population ecology (Lotka 1910; Volterra 1926). This concept of density dependence of predation interactions is engrained into the study of population ecology, as one of the main drivers of biodiversity and species coexistence. The consensus among many ecologists is that abundant species under stable conditions, will – for a given set of environmental conditions - always be limited by either their consumers or by their resources, which will determine a finite population size (Visser 2016; Bowman *et al.* 2017).

Food webs

One way to try to analyze the emergent properties of complex networks of interspecific interactions is by studying their trophic interactions all at once, by defining food webs. In this approach, species within the food web are represented as nodes, connected (i.e. linked) by their trophic interactions (who eats whom), and thereby creating a network. Structural properties of these networks may then be analyzed, including link density (i.e. the average number of feeding interactions per species), connectance (i.e. the relative number of interaction links between species in the network), and trophic distribution properties, which are the relative numbers of primary producers (also called basal species), herbivores, and carnivores (Chapter 2). The organization of a network may then be assessed (e.g. are links randomly or non-randomly organized, which nodes are centralized), and indirect effects of perturbations in one part of the food web to another may be defined. Also, the contribution of all separate links to a species' diet can be studied. For instance, a specialized feeder will eat almost 100% of one food resource, while a more generalist feeder may spread its intake among multiple resources,

creating more, but weaker links. Although extremely laborious, this approach generates much information about energy transfer and nutrient flows throughout the food web.

Important steps have also been taken to study entire food webs in order to gain insight into the way these networks are structured, and the way this influences ecosystem stability. Analysis of food webs provides evidence that they seem to be non-randomly structured (Deangelis 1975), and that simple rules seem to govern their structure and dynamics (Williams & Martinez 2000; Brose et al. 2006). For instance, trophic interactions seem to be largely dictated by body size (Brose et al. 2006; Berlow et al. 2009), and food webs seem to have limitations to connectedness, chain length and size (Dunne et al. 2002a; Riede et al. 2010). A major pursuit in food web studies is to find indicators for network stability, as scientists hope to predict perturbations in food webs and find early warning signs for food web collapse.

Figure 1. Trophic interactions are at the heart of ecological theory. Illustration provided with the courtesy of Jeroen Helmer, Stichting ARK (www.ark.eu).

Network stability has been linked to larger networks, (Dunne & Williams 2009; Riede *et al.* 2010), higher connectedness (Dunne *et al.* 2002b; Gilbert 2009), compartmentalization (i.e. the existence of sub-food webs which interact more with each other than with the rest of the web) (Stouffer & Bascompte 2011) and the presence of weak links (i.e. links contributing only

for a small part to a species' diet) (Neutel *et al.* 2002). However, to this day, after decades of research, stability concepts are still subject of debate (Jacquet *et al.* 2016; Barabas *et al.* 2017).

Non-trophic interactions

More recently however, interactions other than trophic interactions have also gained attention in ecological network studies, since organisms connect in more complex ways than only by feeding on each other, via other, non-trophic interactions (Fig. 2). Interactions between species that are outside, or partly outside the food web can greatly influence the dynamics of a species. For instance, a plant can not only provide food to a herbivore (i.e. a trophic interaction) but also dramatically affect the physical environment of its inhabitants (i.e. non-trophic interactions), for instance by providing shelter, nesting and settling space to many organisms from all kingdoms of life (Bruno *et al.* 2003). Apart from being commensalistic (e.g. via habitat provisioning (Navarrete-Heredia 2001; Callaway *et al.* 2002; Robinson *et al.* 2017)), non-trophic interactions can also competitive or mutualistic in nature. For example, when interference competition occurs, a species uses non-trophic resources (e.g. water, space, light) at the expense of another species, while non-trophic forms of mutualisms benefit both species.

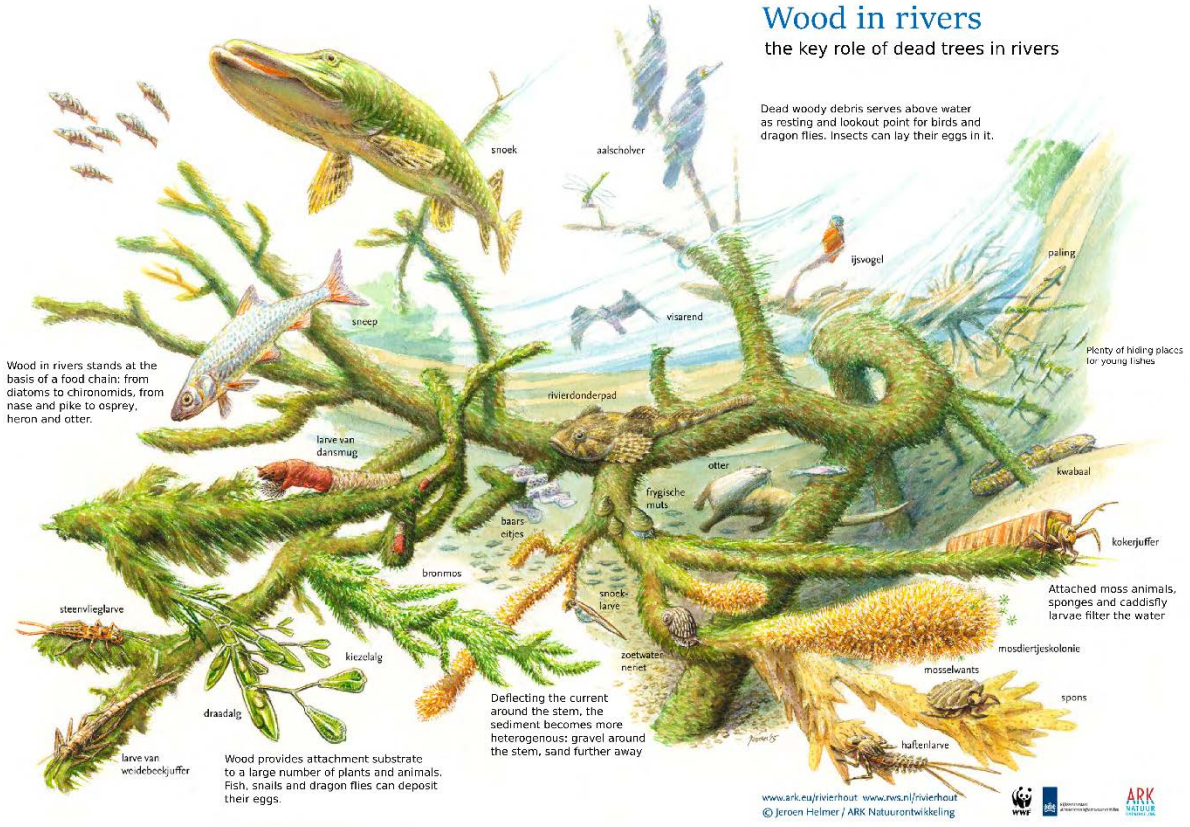

Figure 2. Tree roots and dead trees generate many non-trophic interactions in freshwater systems. They provide shelter, settling and nesting space, deflect shear stress and indirectly concentrate nutrient resources. Illustration provided with the courtesy of Jeroen Helmer, Stichting ARK (www.ark.eu).

Swollen-thorn Acacia trees (*Acacia cornigera)*, for example, provide ants (*Pseudomyrmex ferrugineus*) with housing, while the ants protect the tree from herbivores (Rojas-Soto *et al.* 2014). Note, however, that often multiple interactions occur simultaneously between organism, often yielding a mix of trophic and non-trophic interactions. For instance, when animals disperse plant seed by eating fruit, the resulting relation is mutualistic, with both trophic and non-trophic components (Chapter 5).

Foundation species

Probably all species are involved in non-trophic interaction with other species on some level, but some species play a vital role, influencing the structure and functioning of entire ecosystems. One type of such influential species is called foundation species.

Foundation species are large or spatially dominant sessile organisms (Dayton 1972), also sometimes referred to as autogenic ecosystem engineers (Jones *et al.* 1994) that facilitate many other species. Many types of organisms can be considered foundation species: trees, seagrass, aquatic plants, coral, mussel beds etc. (Jeppesen *et al.* 1992; Bertness & Callaway 1994; Bruno *et al.* 2003; Coker *et al.* 2014). Foundation species are thought to facilitate other species and enhance biodiversity by one of the following three mechanisms: mitigation of environmental stressors, habitat provisioning and the concentration of indirect food resources such as epiphyton or external organic matter (Bruno *et al.* 2003; Govenar 2010). The concept of facilitation is defined as one species altering the growth, survival or reproduction of another species by altering the environment (Bertness & Callaway 1994). Although it always indicates a positive outcome for the facilitated species, the outcome for the facilitator itself is rarely described (Bronstein 2009).

Through their body tissues and physical structure, foundation species modify the landscape and increase structural complexity. Apart from recent observations and experiments, it is also supported by paleoecological studies showing that habitat complexity not only upholds, but also generates biodiversity (Kovalenko *et al.* 2012). Habitat complexity initiates many non-trophic effects which are thought to underlie the increase in biodiversity. For instance, habitat provisioning can cause many non-trophic interactions such as settlement substrate, nursery interactions and temporal uncoupling of predator-prey interactions. Moreover, habitat modification is thought to increase niche creation by increasing environmental gradients and microhabitats (Kovalenko *et al.* 2012). Furthermore, foundation species also often reduce physical or physiological stress (Bruno & Bertness 2001). Mussel beds, seagrass beds, mangrove forests, aquatic vegetation and salt marshes have all been well described for mitigating wave action and shear strength, providing very important coastal defense structures worldwide (Bruno & Bertness 2001; Halpern *et al.* 2007). However, also in more moderately

stressed ecosystems foundation species can be found, where their habitat-provisioning role by providing physical structure may be more important than their role in stress mitigation (Chapter 3). Lastly, foundation species are suggested to concentrate resources such as algae or organic matter (Chapter 2 and 3), and may therefore indirectly provide food sources.

Detangling and integrating trophic and non-trophic interactions

Olff (2009) and Kefi (2012) pointed out that studies on ecological networks often limit themselves to food webs, or a very restricted set of other interactions such as pollinator networks. Non-trophic interactions, on the other hand, have been poorly studied and categorized. Kefi (2012), among many others, suggests to integrate non-trophic and trophic interactions in multilayer network models to further our understanding of ecological networks and the mechanisms that shape them (Olff *et al.* 2009; Kefi *et al.* 2012; Sanders *et al.* 2014; Pilosof *et al.* 2017).

Until now, however, studies trying to integrate multiple interaction types in a single framework in an empirical setting remain scarce (Kefi *et al.* 2015). Reasons for this lack of empirical work are the complexity of the task, and the lack of understanding of the strength of all interactions and the relative contribution and interplay between trophic and non-trophic interactions. The very few studies that have addressed this issue suggest that food webs may be strongly influenced by non-trophic interactions (Kefi *et al.* 2015; Miller *et al.* 2015; van der Zee *et al.* 2016). However, since all these studies were done in marine ecosystems and are still all based on theoretical or correlative data, it remains to be seen how general these conclusions are.

Furthermore, it remains unclear how much of their facilitating effects are driven by purely non-trophic interactions and how much is still trophically driven. To our knowledge experimental studies trying to separate the trophic and non-trophic influences of foundation species remain scarce. Large scale generalizing studies on the overall effects of non-trophic interactions are necessary to take the first steps towards a more integrative understanding of species interactions (Chapter 2 and Chapter 5). Furthermore, small scale studies, shedding light on the contributions of non-trophic/trophic species interactions (Chapter 3 - 5) would help to elucidate differences in species dynamics in space and time.

In my thesis, I have explored different approaches, including fieldwork, experimental manipulation and modeling, to get a grip on the relative contributions of trophic and non-trophic interactions of foundation species, with individual facilitated species and the entire facilitated community (Fig. 3).

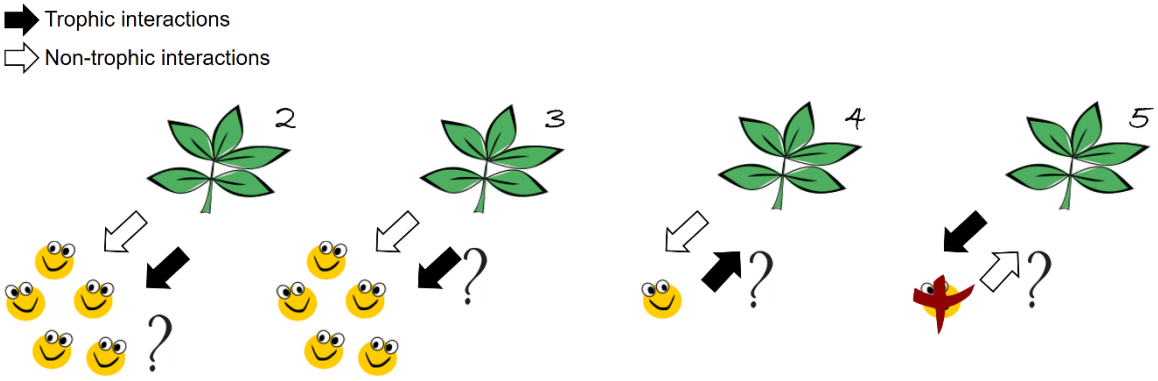

Figure 3. Graphical representation of research questions addressed in the chapters of this thesis. In Chapter 2 I studied the effect of foundation species on the facilitated food web. In Chapter 3 I experimentally studied whether these effects were trophic or non-trophic. In Chapter 4 I studied the long-term trophic effect of a facilitated species on the foundation species. In Chapter 5 I experimentally studied the contribution of a trophic versus a non-trophic interaction in a seed-disperser system.

Objectives and outline of this thesis

In this thesis, I aim to 1) assess the effect of foundation species on the food web, and 2) disentangle the roles of trophic and non-trophic, reciprocal interactions between foundation species and their associated community. To this end, I performed (1) a field survey across multiple ecosystems to elucidate how foundation species change the overall structure of food webs (Chapter 2, Fig. 3), followed by (2) a community-level experimental study to disentangle to what extent foundation species affect their associated community through trophic and non-trophic interactions (Chapter 3, Fig. 3), and (3) two case studies exploring the trophic versus non-trophic effects of facilitated species on the dominant foundation species (Chapter 4,5, Fig. 3).

Specifically, in **Chapter 2** I carried out an in-depth analysis, across different ecosystem types, of emergent patterns in food webs facilitated by foundation species and examined whether this could be generalized across ecosystems. Also, I described whether the changes that occur in the food webs may be trophically or non-trophically driven. Moreover, this study clarifies whether specific species groups are facilitated by foundation species or whether these facilitated species are distributed randomly trough the food web.

Spanish moss (*Tillandsia usnoides*, hereafter Spanish moss), a foundation species in Chapter 2, is considered an important foundation species, facilitating many invertebrate species and even some mammal species. In **Chapter 3**, I use this system as a model to experimentally study the contribution of trophic versus non-trophic interactions of this foundation species on community as a whole. Specifically, I studied how much of the facilitative effect of Spanish

moss festoon on the associated community and the separate feeding guilds can be explained by habitat structure/non-trophic interactions and how much is contributed by food provisioning. Furthermore, I have tested the effect of increased plant size on these facilitative effects.

In **Chapter 4**, I present the outcome of a study focusing on the interactions that Spanish moss (a secondary foundation species) has with its host, the primary foundation species *Quercus virginiana* (hereafter oak). As an epiphyte, Spanish moss depends on the oak for structure. However, it remains unclear thus far whether the oaks benefits or suffers from the presence of Spanish moss by means of their nutrient resource distribution. Here, I compared the nitrogen inputs and outputs in this nitrogen limited system to test whether Spanish moss was restricting or supplementing to the nutrient budget of the oak.

In **Chapter 5**, I show an experimental study of the complex interactions between the abundant tropical palm species *Attalea butyracea* (foundation species) and its facilitated species, seed dispersing but also granivorous rodents. In this chapter I show the interactive effects of disperser-breakdown by hunting on the population dynamics of *Attalea* and how the interaction with density dependent enemies changes the life history of the palm species.

Finally, in **Chapter 6**, I synthesize my work, answer the main research questions, and discuss the results of my work in relation to its advancements of the current scientific knowledge of trophic and non-trophic interactions in foundation species dominated ecosystems.

2. Foundation species enhance food web complexity through non-trophic facilitation

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Abstract

Food webs are an integral part of every ecosystem on the planet, yet understanding the mechanisms shaping these complex networks remains a major challenge. Recently, several studies suggested that non-trophic species interactions such as habitat modification and mutualisms can be important determinants of food web structure. However, it remains unclear whether these findings generalize across ecosystems, and whether non-trophic interactions affect food webs randomly, or affect specific trophic levels or functional groups. Here, we combine analyses of 58 food webs from seven terrestrial, freshwater and coastal systems to test (1) the general hypothesis that non-trophic facilitation by habitat-forming foundation species enhances food web complexity, and (2) whether these enhancements have either random or targeted effects on particular trophic levels, functional groups, and linkages throughout the food web. Our empirical results demonstrate that foundation species consistently enhance food web complexity in all seven ecosystems. Further analyses reveal that 15 out of 19 food web properties can be well-approximated by assuming that foundation species randomly facilitate species throughout the trophic network. However, basal species are less strongly, and carnivores are more strongly facilitated in foundation species' food webs than predicted based on random facilitation, resulting in a higher mean trophic level and a longer average chain length. Overall, we conclude that foundation species strongly enhance food web complexity through non-trophic facilitation of species across the entire trophic network. We therefore suggest that the structure and stability of food webs often depends critically on non-trophic facilitation by foundation species.

Introduction

Food webs and the feeding interactions they consist of have long been the focus of studies aiming to understand the complexity and stability of ecological communities (Elton 1927). There is a long tradition of studying individual consumer-resource (or 'trophic') interactions across the different species that make up a food web and describing the structure of these trophic networks. Collectively, this work has demonstrated that the properties of the trophic network itself, such as the number of species and links, connectance (realized fraction of all possible links), compartmentalization (also referred to as modularity), and the strength of trophic interactions, are important determinants of overall food web stability and robustness (Montoya, Pimm and Sole 2006, Neutel, Heesterbeek and de Ruiter 2002, Dunne, Williams and Martinez 2002, Saint-Beat et al. 2015, Stouffer and Bascompte 2011, Thebault and Fontaine 2010). Furthermore, by extension, these findings indicate that changes in individual trophic interactions have the potential to cascade through the network, thereby destabilizing the entire food web and the corresponding ecosystem (Kuiper et al. 2015, Paine 1969).

Although food webs (i.e. trophic networks) are intensively studied paradigmatic examples of ecological networks (Allesina, Alonso and Pascual 2008, Pimm 1979), organisms do not only interact through feeding interactions. Non-trophic interactions such as habitat modification, mutualism or competition for space have been suggested to indirectly affect food web topology and trophic dynamics by affecting the species in the network and the strength of trophic links (Kefi et al. 2015, van der Zee et al. 2016, Sanders et al. 2014). Although numerous recent theoretical studies have therefore emphasized the urgency to integrate trophic and non-trophic interactions in ecological network analyses (Olff et al. 2009, Kefi et al. 2012, Sanders et al. 2014, Baiser, Whitaker and Ellison 2013, Berlow et al. 2004, Pilosof et al. 2017), empirical studies that do so remain scarce. Indeed, the few empirical studies that did address this knowledge gap suggest that food web structure (i.e. network topology) can be strongly influenced by species interactions outside the trophic network (van der Zee et al. 2016, Kefi et al. 2015, Christianen et al. 2017). However, as these studies only include coastal systems and their number is very limited, it remains unclear to what extent these findings can be generalized across ecosystems. Moreover, whether non-trophic interactions typically affect specific species, trophic levels, or functional groups within the food web, or, alternatively, indiscriminately mediate species and their trophic interactions throughout the network has yet to be resolved. While multiple studies suggest that sessile species with a generally low trophic level benefit more than others from non-trophic facilitation (Baiser et al. 2013, Miller, Page and Reed 2015), other work suggests that facilitation also benefits higher trophic levels and more mobile species (van der Zee et al. 2015, Angelini and Silliman 2014, Christianen et al. 2017).

In this study, we test the general hypothesis that foundation species – spatially dominant habitat-structuring organisms (see e.g. (Angelini et al. 2011, Govenar 2010, Dayton 1972)) – modify food webs by enhancing their size (indicated by species number) and complexity (indicated by link density) via facilitation of species, regardless of ecosystem type. Additionally, we test that any change in food web properties caused by foundation species occurs via random facilitation of species throughout the entire food web or via targeted facilitation of specific species that belong to certain trophic levels or functional groups. Although foundation species are part of the food web like any other species (e.g. as prey or predator), numerous studies have shown that they strongly facilitate the associated community by creating new habitat and alleviating physical stress (Filazzola et al. 2017, Miller et al. 2015, Angelini and Silliman 2014, Reid and Lortie 2012, Jones et al. 2010, van der Zee et al. 2015, van der Zee et al. 2016, Bertness et al. 1999, Kefi et al. 2015). This form of non-trophic facilitation by foundation species has been found to occur across a wide range of ecosystems and environmental conditions (Bruno, Stachowicz and Bertness 2003, Bertness and Callaway 1994). In harsh coastal zones, corals, kelps, mussels, oysters, seagrasses, mangroves, and salt marsh plants facilitate organisms by attenuating currents and waves, providing aboveground structure for shelter and attachment, concentrating nutrients, and/or reducing desiccation stress during low tide exposure (Bertness and Callaway 1994, Angelini et al. 2011). In more benign systems, foundation species such as the trees in a forest, shrubs and grasses in savannahs, and macrophytes in freshwater systems, have also been found to play a major habitat-structuring role (Bruno et al. 2003, Bertness and Callaway 1994, Ellison et al. 2005, Jeppesen et al. 1992). Ultimately, all foundation species increase habitat complexity and availability, thereby partitioning and enhancing the niche space available to other species (Bruno et al. 2003, Bulleri et al. 2016).

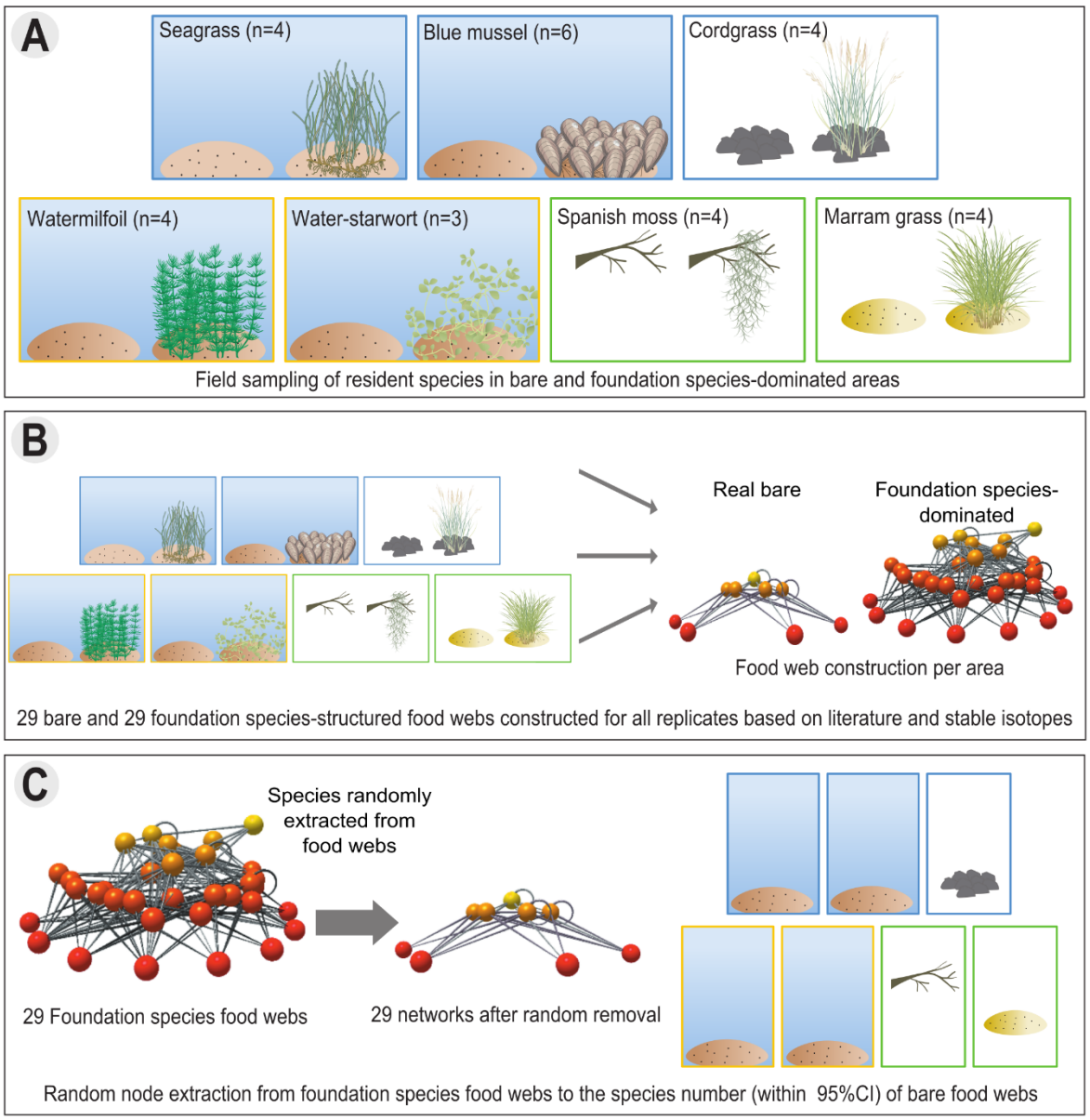


Figure 1. Illustration of the methods. (A) Seven ecosystems (including coastal (blue border), freshwater (yellow border) and terrestrial (green border)) were sampled (B) food webs were constructed, for both bare and foundation species-dominated replicate areas. (C) Finally, from each foundation species structured-food web we randomly removing nodes (i.e. species) until the species number matched the species number of the bare food webs within the 95% CI per ecosystem. Some of the symbols used in this figure were provided with the courtesy of Tracey Saxby, Dieter Tracey, Kim Kraeer and Lucy van Essen-Fishman, IAN Image Library (ian.umces.edu/imagelibrary/).

To test whether foundation species consistently increase food web size and complexity as hypothesized, we sampled species from areas dominated by foundation species with well-documented effects on their habitats (29 food webs) and from nearby bare, unmodified areas (29 food webs) in seven ecosystem types, including two terrestrial, two freshwater and three marine ecosystems. For each sampled area, we constructed the food web from the retrieved species using literature surveys, stable isotope analyses, and mixing models, after which we

compared the properties of the bare and foundation species-dominated food webs. Next, we randomly removed species from each constructed foundation species-dominated food web to the number of species found in neighbouring bare areas (Fig 1). By comparing the properties of these simulated, random-removal networks to those of the real food webs, we investigate whether the observed food web modifications by foundation species arose from random or selective facilitation of trophic levels or functional groups across the trophic network

Results

Despite large differences between all seven ecosystems in terms of environmental conditions and trophic network structure, we discovered that food web size and complexity, expressed as species richness and link density, respectively, were consistently enhanced in foundation species-dominated areas compared to food webs in nearby bare areas (Fig 2, Table 1 and S1 Fig). Specifically, species richness was on average 2.1 times higher, and link density increased 1.6 times in the presence of foundation species. Connectance – the realized fraction of all possible links – showed an opposite response and decreased 0.75 times. Out of the 19 food web metrics measured (full description of calculated metrics in S1 Table), 13 differed significantly between bare and foundation species-dominated areas, suggesting pervasive modifications to food web structure (Table 1). For instance, trophic distribution properties showed that the fraction of basal species decreased by 30%, the intermediate species fraction increased by 40%, and the top species fraction remained unchanged in response to foundation species presence. Furthermore, the average shortest chain length to a basal species increased by 30%, while the average trophic level and average path length between species (measure of energy transfer efficiency (Avin et al. 2015)) increased by 10%. Vulnerability – i.e. the number of consumers for each species – did not differ significantly for foundation species compared to other species, indicating that they are not consumed any more or any less compared to other species in the network (S2A Fig). We also found that the total number of trophic links to foundation species was almost half compared to the average number of trophic links per species in the rest of the network (S2B Fig). Moreover, this effect remained even when comparing only the number of links of basal foundation species versus other basal species (S2C Fig).

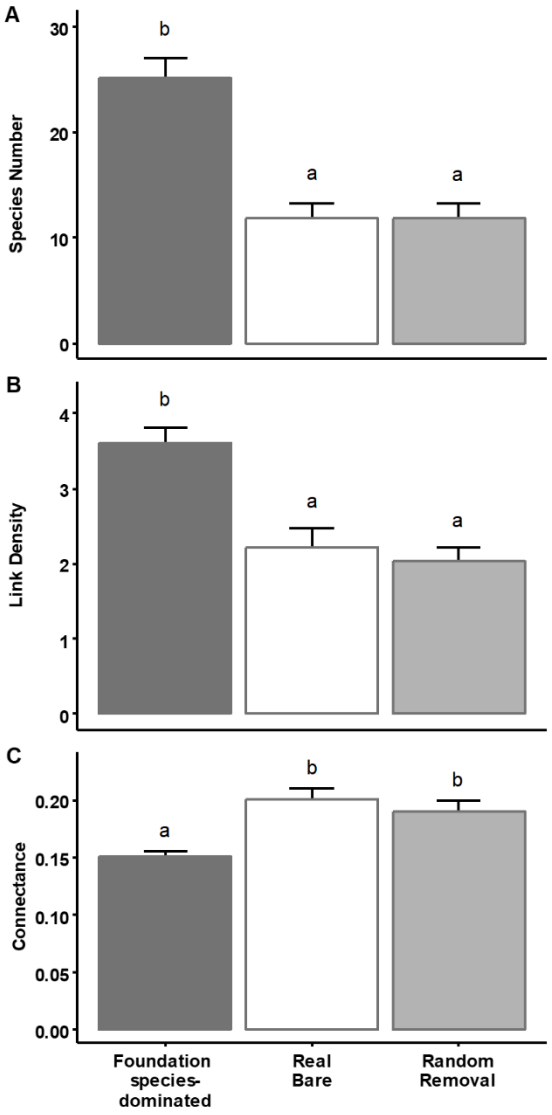


Figure 2. The presence of foundation species consistently changed food web properties (mean±SE) across ecosystems. Including (A) Species richness, (B) Link density, (C) Connectance. The random removal of nodes created networks which corresponded well with the properties of real bare food webs.

Table 1. Changes in food web properties between foundation species-dominated food webs, food webs from a bare area and random removal networks, and the result summary from the LMEs.

Metrics	**AVERAGE ± SE[a]**			**Statistics[b]**			
	Foundation species dominated (FS)	Real Bare (BA)	Random Removal networks (RR)	F	p	Posthoc (FS, BA, RR)	Ecosystem
Species Number	25 ± 1.9	12 ± 1.4	12 ± 1.4	80	***	b, a, a	***
Link Density	3.6 ± 0.21	2.2 ± 0.24	2 ± 0.18	58	***	b, a, a	***
Connectance	0.15 ± 0.005	0.2 ± 0.009	0.19 ± 0.01	20	***	a, b, b	***
Vulnerability	3.5 ± 0.2	2.1 ± 0.23	1.9 ± 0.18	62	***	b, a, a	***
Generality	3.6 ± 0.21	2.2 ± 0.24	2 ± 0.18	58	***	b, a, a	***
Links	6.9 ± 0.41	4.1 ± 0.46	3.7 ± 0.35	62	***	b, a, a	***
Top fraction	0.29 ± 0.018	0.31 ± 0.023	0.36 ± 0.021	3.5	*	a, ab, b	ns
Intermediate fraction	0.47 ± 0.02	0.34 ± 0.044	0.37 ± 0.024	5.7	**	b, a, a	***
Basal fraction	0.24 ± 0.019	0.35 ± 0.032	0.26 ± 0.024	13	***	a, b, a	***
Herbivore fraction	0.26 ± 0.033	0.28 ± 0.031	0.24 ± 0.029	1.1	ns	a, a, a	***
Omnivore fraction	0.26 ± 0.037	0.24 ± 0.04	0.21 ± 0.035	2.8	ns	a, a, a	***
Carnivore fraction	0.24 ± 0.027	0.14 ± 0.028	0.29 ± 0.03	18	***	b, a, b	***
Cannibal fraction	0.14 ± 0.015	0.15 ± 0.023	0.17 ± 0.02	1.1	ns	a, a, a	***
Chain Length	1 ± 0.043	0.79 ± 0.056	1 ± 0.049	19	***	b, a, b	***
Trophic Level	2.1 ± 0.06	1.9 ± 0.085	2 ± 0.069	17	***	b, a, b	***
Max. Similarity	0.68 ± 0.017	0.57 ± 0.046	0.52 ± 0.05	8.1	***	b, a, a	***
Clustering	0.29 ± 0.034	0.26 ± 0.047	0.25 ± 0.037	0.87	ns	a, a, a	***
Path Length	1.9 ± 0.023	1.7 ± 0.059	1.7 ± 0.048	10	***	b, a, a	***
Compartmentalization	0.23 ± 0.009	0.19 ± 0.019	0.2 ± 0.019	2.8	ns	a, a, a	***

[a] Foundation species-dominated food webs (FS), Real Bare food webs (BA), Random removal networks (RR).

[b] Effects were tested in a mixed model with the ecosystems as random factor. ***: p<0.001, **:p<0.01, *:p<0.05, ns: not significant

Random removal of species from the foundation species-dominated food webs until the species number matched the numbers found in bare areas simplified trophic networks, and altered most food web properties such that they resembled those from the bare areas we sampled (Table 1 and Fig 2). Moreover, when we combined the food web metrics from foundation species-dominated, real bare, and random removal food webs in a Principle Components Analysis (PCA), we discovered that most of the variation (96.6%) in the metrics could be condensed onto a single Principle Component axis, PC1 (Fig 3, PC2 explained only another 1.6% of the variation). PC1 clearly differentiated real bare, and foundation species-structured food webs ($F_{2, 78}$=79.9, $p<0.0001$), but did not differentiate between real bare and random removal food webs (Fig 3B). This indicates that, when analyzing the overall repsonse of food web metrics, networks created by random species removal corresponded well with those observed in the real bare areas.

Our PCA results are supported by more in-depth comparative analyses of individual metrics. Whereas 13 out of 19 metrics included in our analyses differed significantly between food webs from foundation-dominated and bare areas, only 4 out of 19 metrics deviated significantly when comparing real bare and random removal food webs (Table 1 and S1 Fig). Interestingly, these four metrics – the basal fraction, number of carnivores, average shortest chain length and average trophic level – also showed the strongest correlation with PC2 from the PCA (Fig 3A). Specifically, we found that, after randomly removing species from real, foundation species-dominated food webs, the fraction of basal species was significantly lower, while the number of carnivores, average chain length, and average tropic level were higher compared to real bare area food webs.

Discussion

Multiple theoretical studies have suggested that habitat-modifying organisms (i.e. foundation species, ecosystem engineers) significantly enhance food web size (i.e. species richness) and complexity (i.e. link density) by affecting species and links through non-trophic interactions (Olff et al. 2009, Sanders et al. 2014, Baiser et al. 2013, Kefi et al. 2012). Although this hypothesis was recently supported by two empirical studies of coastal ecosystems (van der Zee et al. 2016, Christianen et al. 2017), it remained unclear whether these findings could be generalized to other ecosystems. By comparing foundation species-dominated habitats with nearby bare habitats where these foundation species ware absent, we demonstrate that their presence consistently increased food web size and complexity across seven terrestrial, freshwater and coastal ecosystems. We found that, in all seven investigated ecosystems, facilitation by foundation species increases the number of species, and the number of links per species, while decreasing link saturation (connectance). Moreover, our analyses provide

support for the hypothesis that non-trophic facilitation by foundation species facilitates associated species throughout the trophic network.

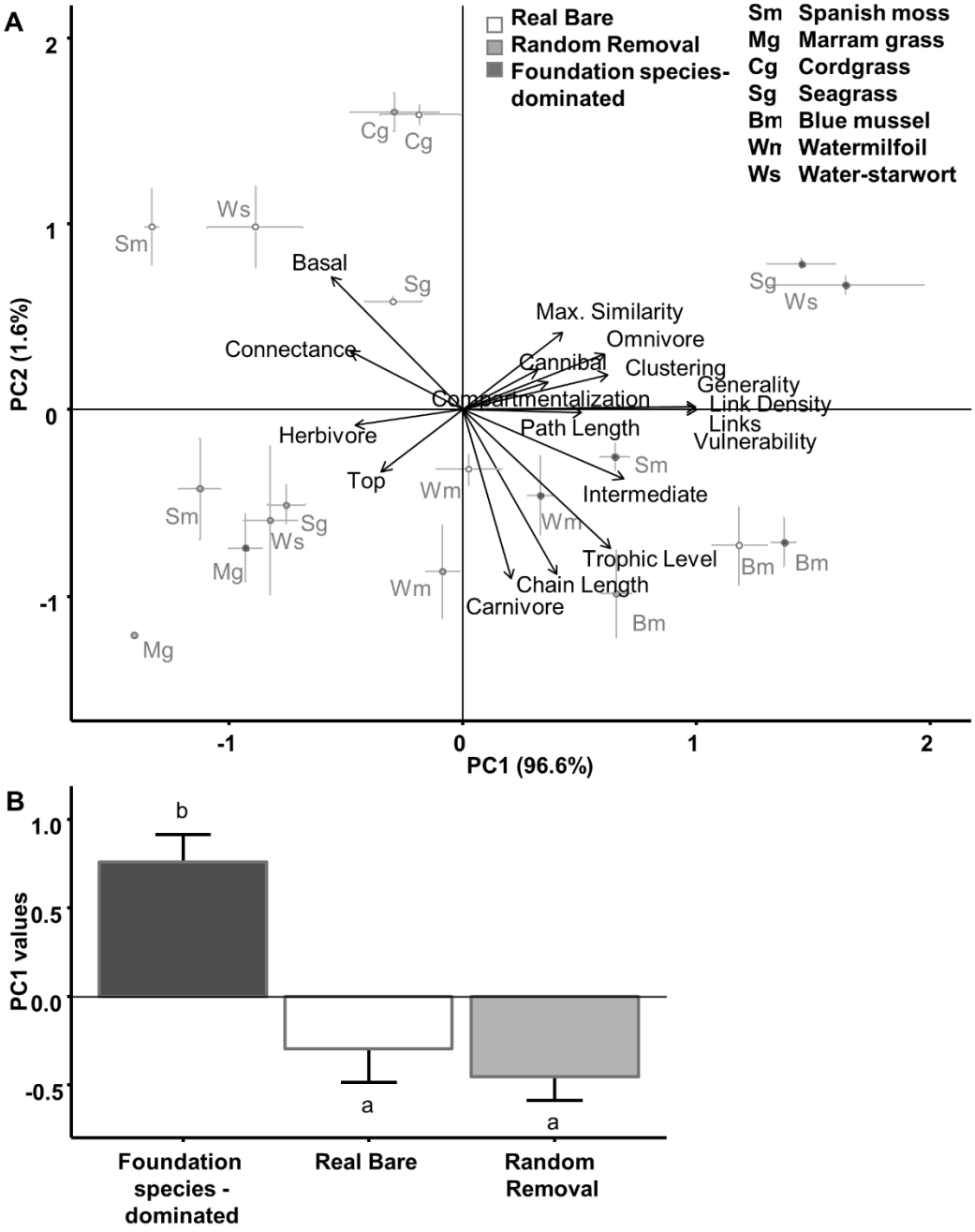


Figure 3. PCA Axis 1 clearly differentiated between bare and Foundation species-dominated, but not between bare and random removal networks (A) Averaged PCA values (mean±SE) of all food web metrics describing both field and simulated food webs of foundation species-dominated and bare areas. Arrows are projected food web metrics (total variation 1090, axis 1: 96.6%, axis 2: 1.6%). (B) Scores of Principle Component axis 1 explained by real bare versus foundation species-dominated ($p < 0.0001$), and real bare versus random removal networks (ns).

As foundation species in our study were themselves also part of the food web, the observed changes in food web complexity and properties could theoretically have resulted from their trophic role instead of non-trophic facilitation. However, although the trophic network did increase in size in the presence of foundation species, the number of species feeding on foundation species - i.e. their vulnerability - did not differ significantly from the number of species feeding on other species in the network (S2A Fig). Moreover, we found that the number of trophic links to foundation species was in fact much lower compared to the average number of trophic links of other species in the network even when comparing only basal species (S2B and S2C Figs). Although foundation species with only few trophic links could theoretically still be important in the food web by serving as a vital food source for highly connected species, this is unlikely as a large number of studies have revealed that the palatability of foundation species in general can be considered rather low (van der Zee et al. 2016, Miller et al. 2015, Jaschinski, Brepohl and Sommer 2011, Wright et al. 2014). These results suggest that trophic facilitation on its own is not a likely explanation for the observed enhancements of food web complexity by foundation species. Instead, non-trophic facilitation would seem to be the main driver, corroborating a large body of earlier work showing that habitat modification and stress amelioration by foundation species is critical to their enhancement of species richness (van der Zee et al. 2016, Angelini et al. 2011, Bertness and Callaway 1994, Bertness et al. 1999, Angelini and Silliman 2014, Christianen et al. 2017). Thus, our empirical work provides compelling cross-ecosystem evidence for the hypothesis that non-trophic facilitation by foundation species, rather than their trophic role, can be an important driver of food web structure (Olff et al. 2009, Baiser et al. 2013, Kefi et al. 2012, Kefi et al. 2015, van der Zee et al. 2016).

The underlying mechanisms by which foundation species non-trophically facilitate associated species may differ widely across ecosystems, including those we investigated. Simple provisioning of attachment substrate and three-dimensional structure has been found to be an important mechanism of facilitation by all foundation species (Bruno et al. 2003, Bertness and Callaway 1994, Dayton 1972, Kovalenko, Thomaz and Warfe 2012). In addition, species may also be benefit from foundation species because the foundation species concentrates critical resources (e.g. water, detritus), or mitigates physical stress resulting from currents, waves, wind, sediment instability, drought or high temperatures (Bertness et al. 1999, van der Zee et al. 2016, Christianen et al. 2017, Wright and Gribben 2017). Furthermore, indirect facilitation through trophic pathways is also possible. For instance, foundation species may trap or accumulate nutrients, detritus, and other resources (S3 Fig), or mediate a trophic cascade in which predators depend on prey that is non-trophically facilitated by the foundation species (Jaschinski et al. 2011, Bologna and Heck 1999).

Our analyses reveal that random removal of species from foundation species-dominated food webs yielded food webs very similar to bare areas (Fig 3). Yet, despite their similarities, four

metrics – the basal species fraction, carnivore fraction, average chain length, and average trophic level –deviated, suggesting that non-trophic random facilitation can explain the observed food web modifications to a large extent, but not completely. In foundation species-dominated food webs, the number of basal species is relatively lower, while the number of carnivores is higher than predicted based on random facilitation. Foundation species can compete for space with larger sessile species that occur at the base of the food web, possibly explaining the relatively reduced representation of basal species in foundation species-dominated food webs (Kefi et al. 2015). Furthermore, carnivores may be particularly dependent on habitats that support high densities of prey and which are characterized by low physical stress – conditions created by the foundation species (Klecka and Boukal 2014, Zhao et al. 2016), resulting in their overrepresentation in foundation species-dominated food webs. As a result of the relatively higher number of carnivores that occur at higher trophic levels, the average chain length and average trophic level likely tends to be higher than expected from random facilitation in these conditions.

One important explanation for why foundation species facilitate other species that occur throughout the entire food web is that they provide three-dimensional structure (Bruno et al. 2003), thereby enhancing niche availability and complexity (e.g. via the creation of an epi-benthic next to an endo-benthic community). Earlier work showed that random removal of species has a lower impact on food web structure compared to the removal of specific species or trophic groups. Specifically, studies by Sole and Montoya (2001), Dunne *et al.*(2002b) and Mulder *et al.* (2012) all found that random removal of species leads to less secondary extinctions and a higher robustness of food webs compared to a more targeted removal of species. Hence, based on this earlier work, our findings suggest that foundation species, by facilitating species across trophic levels, sustain food webs that are more robust than those that would be generated if they were to facilitate specific trophic levels, feeding guild or functional groups.

Although foundation species may in principle stabilize and enhance the size of food webs through niche creation, this does not necessarily mean that foundation species-supported food webs are more resilient to real-world disturbances. In the foundation species-supported food webs that we analyzed, over 50% of the species appear directly or indirectly dependent on non-trophic facilitation by foundation species (Table 1). This implies that the food webs associated with foundation species are likely very sensitive to disturbances that affect the health and functioning of foundation species. At present, many ecosystems worldwide that are structured by foundation species – including seagrass meadows, salt marshes, coral reefs, peatlands and rainforests – are declining at an alarming rate due to anthropogenic disturbances (Chazdon 2003, Waycott et al. 2009, Bellard et al. 2012, Gedan, Silliman and Bertness 2009, MEA 2005).

Our findings suggest that to preserve complex but stable food webs across ecosystems, it is vital to prioritize the conservation and restoration of the foundation species that support them.

Methods

Study systems

Foundation species are sessile, spatially dominant habitat-modifiers that create physical structures with their own body tissue. Many plants such as seagrasses or trees can be considered foundation species, but many corals and bivalves fit this definition (Dayton 1972, Bertness and Callaway 1994, Bruno et al. 2003, Govenar 2010). These species often facilitate their own growth in a scale dependent manner. This behavior typically results in emergent pattern formation (Gilad et al. 2007, Gilad et al. 2004, Rietkerk and Van de Koppel 2008) with locally clustered areas of foundation species and adjacent bare habitat without foundation species (i.e. rocks or sand).

We sampled 58 food webs from seven different ecosystem types using a consistent methodological approach. Although the abiotic environmental conditions vary widely across these ecosystems, all are typified by the presence of a spatially dominant foundation species that enhances habitat complexity, and mitigates environmental stress (Bertness and Callaway 1994, Bruno et al. 2003, Dayton 1972). Our study included three coastal ecosystems: (1) intertidal seagrass beds dominated by *Zostera noltii* in Banc d'Arguin, (Mauritania), (2) cordgrass-dominated (*Spartina alterniflora*) fringing marshes growing on the cobble beaches of Rhode Island (USA), and (3) intertidal blue mussel beds (*Mytilus edulis*) in the Wadden Sea (the Netherlands). Apart from enhancing habitat complexity, the foundation species of these coastal ecosystems all attenuate current and waves, stabilize the substrate, and provide shelter and attachment for other species (Gutierrez et al. 2003, Donadi et al. 2013, van der Zee et al. 2015, Donker, van der Vegt and Hoekstra 2013). In addition, seagrass and mussels trap large quantities of suspended particles from the water column, whereas cordgrass provides shading and stabilization of cobble stones (Altieri, Silliman and Bertness 2007). We also included two freshwater ecosystems: (1) Watermilfoil (*Myriophyllum alteniflorum*) dominated shallow lakes in a dune slacks with standing water on the Wadden Sea island of Terschelling (the Netherlands), (2) and Water-starwort (*Callitriche obtusangula*) dominated slow flowing streams (Desselse Nete, Belgium). Freshwater macrophytes have been found to facilitate other species by providing structure and shelter against flow stress and predation by fish and apex invertebrate predators (Grutters et al. 2015, Lodge 1991, Verdonschot, Didderen and Verdonschot 2012). Finally, we sampled two terrestrial systems: (1) marram grass-dominated (*Ammophila arenaria*) dunes at Terschelling, and (2) Spanish moss (*Tillandsia usneoides*)-dominated live oaks in Georgia (USA). Marram grass attenuates wind and heat stress (S4 Fig),

while Spanish moss reduces desiccation and predation stress relative to adjacent stretches of bare live oak branch. (Angelini and Silliman 2014)

Sampling

To sample the food web in each foundation species-colonized and bare habitat, we followed the general methodology by Van der Zee et al (2016). Within each ecosystem we sampled two contrasting habitat types: areas dominated by foundation species and bare areas where the foundation species was absent. To minimize differences in underlying environmental conditions, all habitats were sampled in a pairwise manner, with the exception of Banc d'Arguin where this was not possible as habitat modification effects occur at much larger scales compared to the other ecosystems. Instead, to avoid dissimilarities in the underlying conditions due to environmental gradients, all sampling stations were selected based on a random spatial distribution, with similar elevation, distance to the gully, maximum fetch length and Exposure Index—an integrative measure of wave exposure (see (van der Zee et al. 2016)). A full description of the sampling strategy per ecosystem can be found in S1 Text in Supporting Information. In all seven systems we sampled 3 to 6 replicate areas (58 food webs total; Fig 1). For each sampled area, we collected and identified all abundant species that collectively represented at least 95% of the biomass in each habitat.

After collection, all samples were stored in the freezer (-20°C) until further analysis. The samples were then dried to constant weight, either by means of an oven set between 50 and 60 °C, or by means of freeze-drying. Dried samples were homogenized using a hand mill or mill grinder (Retsch, Aartselaar, Belgium). Finally all samples were analyzed in duplicate on an Isotope-ratio Mass Spectrometer (IRMS) (Thermo Scientific, Waltham, Massachusetts, USA) for δ^{13} carbon isotopic signal and δ^{15} nitrogen isotopic signal.

Food web reconstruction

Dichotomous food webs were constructed for each sampled area (Fig 1A). We used scientific literature, databases and expert knowledge to construct a maximized theoretical network that included all possible trophic links for the sampled organisms. To include only ecologically relevant interactions and omit incidental ones, we then constrained each maximized network by removing rare species (i.e. <3 individuals counted in each ecosystem), and highly improbable interactions, for instance those based on size discrepancies between predators and prey based on expert judgement (Kefi et al. 2015, van der Zee et al. 2016). Finally, based on diet reconstruction using stable isotope bi-plots of δ^{13}C and δ^{15}N and Bayesian mixing models (R-package SIAR (Parnell and Jackson 2010)), we further constrained the network by removing improbable trophic links where prey contributed less than 5% to the consumer's diet (van der Zee et al. 2016, Christianen et al. 2017). Food webs were constructed in a dichotomous

n*n matrix, in which the columns represent all n species as predators and the rows represent all n species as prey. Based on these matrices, we calculated a number of widely used food web metrics per replicate food web (Table 1).

Trophic importance of foundation species

To determine the extent to which foundation species trophically affected food web structure, we assessed their trophic contribution relative to those of other species by comparing their vulnerability metric (Table 1) to the average vulnerability of all other species in the food web. High values would indicate that the foundation species is a relatively important food source in the network and may thus strongly affect food web properties via its trophic role. We also determined the number of links of foundation species, and compared it to the average of all other species in the food web ('Links' in Table 1). In addition, as most foundation species are basal species, which do not feed on other species in the food web and may therefore have fewer overall links, we also compared the number of links of basal foundation species to the average number of links of other basal species in the food web.

Random species removal

To test whether observed food web modifications by foundation species arose from random or selective non-trophic facilitation of species, links or levels across the trophic network, we randomly removed species from foundation species-dominated food webs to match the number observed in neighbouring bare systems. Specifically, we pruned the foundation species-structured food webs by randomly removing species, also deleting species that became trophically isolated from all other species (i.e. no remaining feeding links or not connected to any other species) as a result of this random removal procedure. For each food web, we randomly removed nodes until the number of species in the remaining trophic matrix fell within the 95% confidence interval of the average number of species in the corresponding bare plots (Fig 1C). Finally, we calculated all abovementioned food web metrics (Table 1) again for the randomly-constrained model-derived 'bare' food webs. If foundation species facilitate food webs in a random fashion (i.e. the foundation species does not have disproportionate effects on specific species, trophic levels or functional groups), the empirical bare food webs and their properties should be indistinguishable from those of the simulated bare plots after our random pruning procedure.

Statistical analyses

We used Linear Mixed-Effect models (LME) to compare individual food web metrics (listed in Table 1) between food webs from foundation species-dominated areas, bare areas and the random removal procedure (hereafter food web type) Significance was tested using one-way

ANOVA F-tests with a Satterthwaite approximation of the degrees of freedom (package lmerTest in R(Kuznetsova, Bruun Brockhoff and Haubo Bojesen Christensen 2015)), and Tukey posthoc tests to differentiate between food webs type. In these analyses, we used food web type as a fixed factor and ecosystem as a random factor.

Trophic dependency (i.e. average vulnerability) and total number of links of the foundation species versus the average number of links of the other species in that network were analyzed using foundation species or 'other species' as fixed factor and ecosystem as random variable in a LME and an ANOVA F-test. Residuals of all models were checked for normality by Shapiro Wilk's test and a qq-plot and response variables were log-transformed when necessary. Trophic dependency on basal foundation species versus the average number of links of other basal species was analyzed in the same way.

To assess the overall combined response of food web metrics to food web type, we constructed Principal Component analysis (PCA), analyzing the different replicate food webs as samples and food web metrics as variables. Scores of the first Principle Component axis were taken as a composite measure of food web structure and were analyzed with a LME model and tested with a one-way ANOVA F-test using food web type as a fixed factor and ecosystem as random variable to assess the effect of our random removal procedure on overall food web structure.

Acknowledgements

We thank Jan Roelofs for providing background information on the dune slacks and Eva van den Elzen, Laura Govers, Valérie Reijers and the students from the Systems Ecology course for their help with the food web sampling. We thank Jelle Eygensteyn and Paul van der Ven for their technical support, Dick Visser assistance with the graphics, and L. P. M. Lamers for his comments on the manuscript.

Supplementary Materials

Supplementary figures

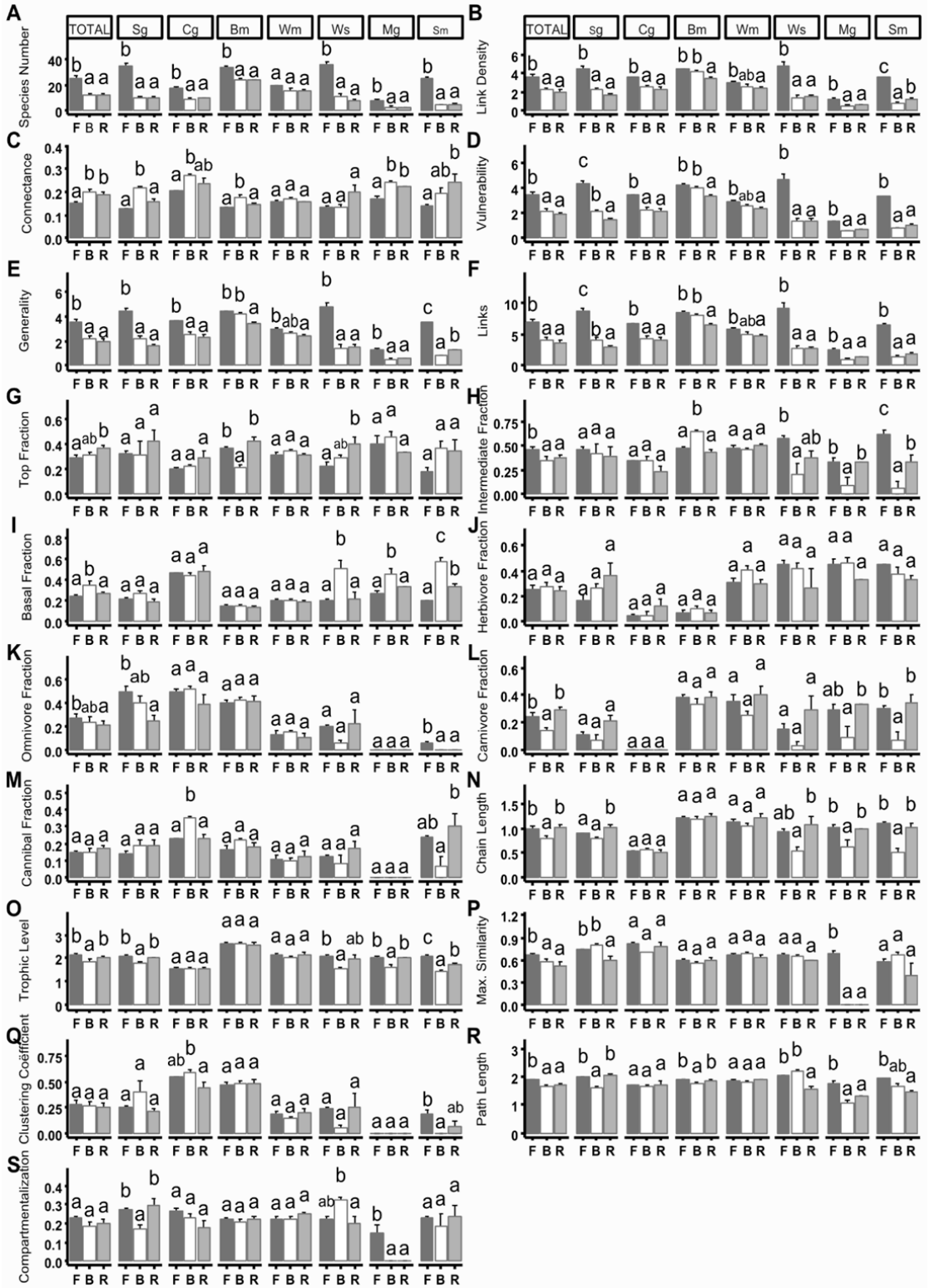


Figure S1. Food web properties averaged and per ecosystem. Properties are arranged in Marine, Freshwater and Terrestrial (Sg: Seagrass, Cg: Cordgrass, Bm: Blue mussel, Wm: Watermilfoil , Ws: Water-

starwort, Mg: Marram grass, Sm: Spanish moss) averaged for Foundation species-dominated food webs (FS), food webs from bare areas (BA) and random removal networks (RR).

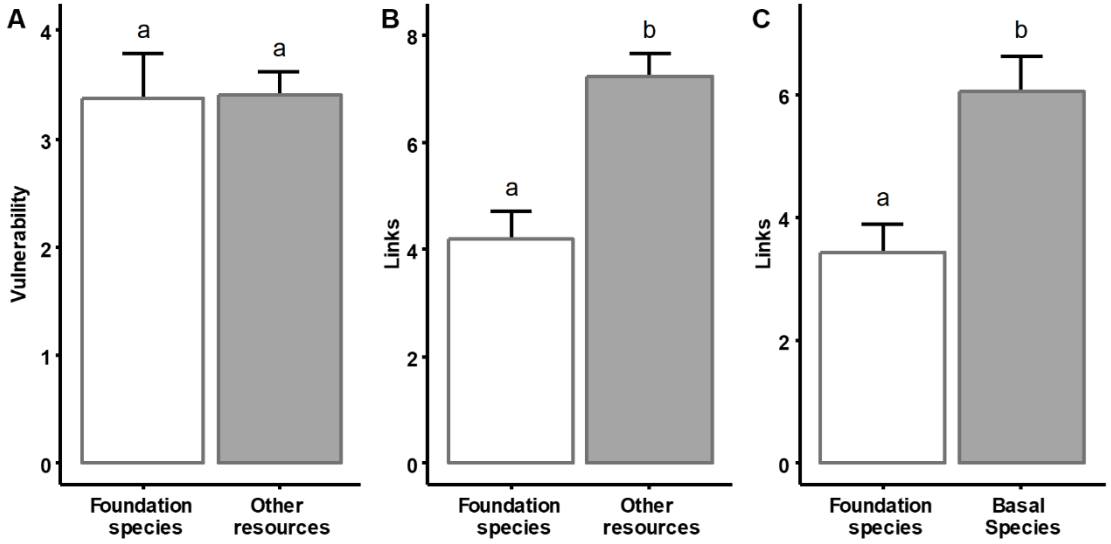


Figure S2 Trophic dependency on foundation species compared to other species in the network. Trophic dependency is not higher for foundation species when expressed as vulnerability (A) the total number of links compared to the other species (B), or the number of outgoing links of basal foundation species versus other basal species (C) for which the number of links equals vulnerability.

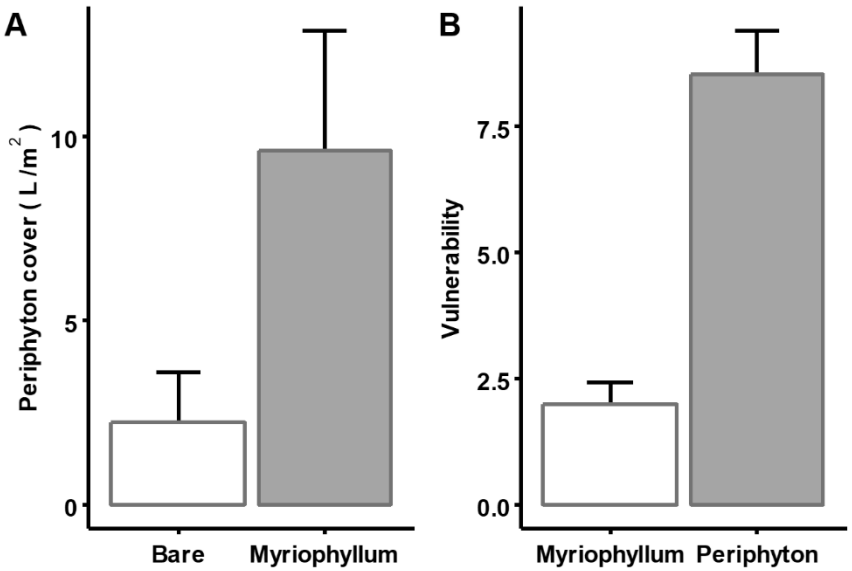


Figure S3. Watermilfoil indirectly stimulates the food web by increasing periphyton availability. (A) Watermilfoil increased periphyton cover. (B) Trophic dependency on periphyton as a food source is much higher than on Watermilfoil.

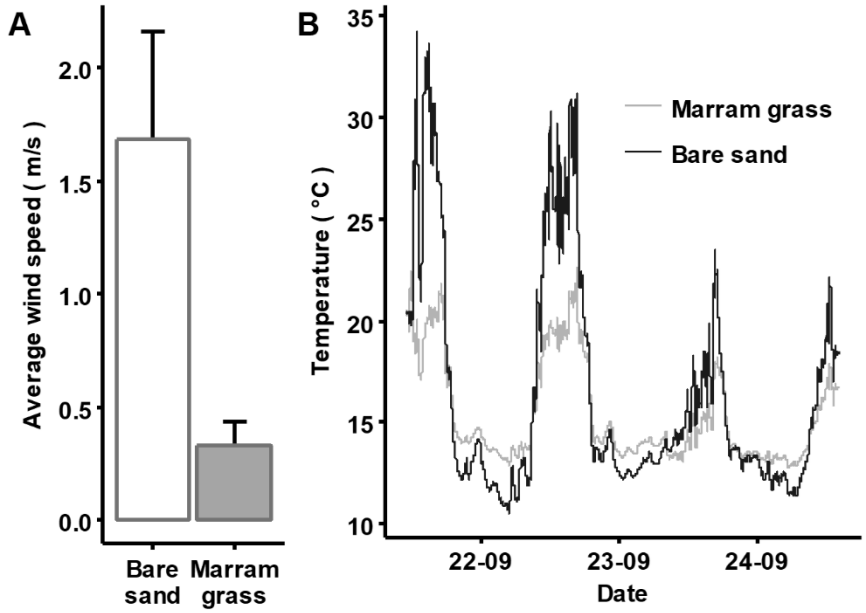


Figure S4. Stress mitigation in Marram grass plots compared to bare sand. Marram grass mitigates (A) wind speed and (B) maximum temperature and temperature variability.

Supplementary tables

Table S1. Food web metrics calculated for all food webs

Metric	Meaning
Species number	Number of trophic nodes in a food web
Link density	Average number of links (predator or prey links) per species
Connectance	Link saturation in the network, Links/Species^2
Generality	Average number of prey/resources per species, not including cannibalism
Vulnerability	Average number of predators/consumers per species
Links	Average number of links (of prey and predators) per species, not including cannibalism
Top species fraction	Fraction of species that are not eaten by other species
Intermediate fraction	Fraction of species that are both a predator and prey
Basal species fraction	Fraction of species that do not eat any other species
Cannibal fraction	Fraction of species that eat from their own node
Herbivore fraction	Fraction of species that solely eat basal species

Metric	Meaning
Omnivore fraction	Fraction of species feeding from both basal species and higher trophic levels
Carnivore fraction	Fraction of species feeding from anything but basal species
Trophic level	Average trophic level
Chain length	Average shortest chain length from any species to any basal species
Maximum similarity	Average maximum Jaccardian similarity within the network, i.e. maximum shared neighbouring nodes between all species pairs.
Clustering coefficient	Clustering coefficient: Average clustering of neighbouring species
Path length	Average shortest path length between all species pairs
Compartmentalization	Also referred to as modularity; it is the division of a network into sub-communities

Supplementary texts

Detailed description of sampling methods per model ecosystem.

Fringing marshes, New England, USA

We randomly selected four replicate plots with similar elevation, distance to the gully, maximum fetch length and Exposure Index at low tide for both bare cobble stone and *Spartina alterniflora*. Within each plot, a 25×25 quadrat was placed to count the number and abundance of resident (i.e., not migrating with the tide) species.

Seagrass meadows, Banc d'Arguin

We established four plots of bare sand and seagrass meadows in 50-m diameter circles during low tide. Within this circle, 4 replicate areas for sediment and (endo)benthos samples were selected. An extensive description of the sampling of both the cord grass and the seagrass can be found in van der Zee *et al.* (2016).

Watermilfoil in a dune lake

In dune lake El Dorado on Terschelling Island, The Netherlands (53°24'N, 5°16'E), we selected bare, sandy patches and patches where Watermilfoil (*Myriophyllum alterniflorum*) covers up to a 100% of the area. We randomly selected 4 replicate plots for each type of habitat and sealed the fauna present in the plot by placing a sampling ring with a diameter of 45 cm. The fauna in the water layer was sampled by sieving all the water within the ring over a 1 mm mesh sieve. The periphyton layer on and sediment samples was taken using a 50 ml-corer. All plant

material in the plot was harvested and macrofauna hiding between the plants were collected later in the lab. Zooplankton and floating algae samples were collected separately for the whole lake. To this end, surface water was filtered over a 200-µm zooplankton net and finally precipitated onto Whatman GF/F filters after which it could be collected by scraping the filter carefully. Zooplankton was collected using a zooplankton net, and subsequently filtered onto Whatman GF/F filters after which it was collected by scraping the filter. All samples were stored at -20˚C until further processing. Fauna species were identified in the lab to the lowest feasible level, mostly to family level. All samples were then rinsed with demineralized water, freeze-dried and homogenized using a ball grinder (Retch). Homogenized samples were weighed in tin cups and analyzed for carbon and nitrogen stable isotope composition on an isotope ratio mass spectrometer (IRMS, Thermo Scientific).

Marram grass in the dunes of Terschelling, The Netherlands

In the dunes of the Terschelling Island in The Netherlands (53°25'N, 5°24'E), we randomly selected 4 replicate plots of bare, sand dune habitat and within the marram grass tussocks (*Ammophila arenaria*), which were covered up to 90% with marram grass. All plots were sampled on the same dune, no more than 50 meters apart.

We sampled flying insects in the plots by sweeping a fly net back and forth fifteen times. Then we manually collected the ground dwelling fauna by placing a sampling ring with a diameter of 45 cm around the plot. We collected all marram grass present in a plastic bag which was later sorted for fauna hiding within the rolled up of the grass.

A sample of 5 liter taken from the sediment up to 10 cm deep and put through a 5 mm mesh sieve. Also 1 liter from this 5 liter was sieved at a mesh size of 1 mm. No subterrestrial species were found in these samples. Invertebrates were then identified, mostly to species level. All samples were dried at 60°C until stable weight and homogenized using a ball grinder (Retch). Homogenized samples were weighed in tin cups and analyzed for carbon and nitrogen stable isotope composition on an isotope ratio mass spectrometer (IRMS, Thermo Scientific).

Spanish moss at Sapelo Island, Georgia USA

Spanish moss food web samples were collected on Sapelo Island, Georgia USA (31°25'N 81°16'W). In 4 trees of Southern Live oak (*Quercus virginiana*), we randomly sampled the food webs of both 8 paired replicate ~50 cm long bare branches and branches with a large 'festoon' of Spanish moss (*Tillandsia usnoides*). The 8 collected samples were pooled to construct one bare food web and one Spanish moss food web per Live oak.

An airtight bag was used to enclose all invertebrates in a volume of ~40 cm^3 around the branch. Mobile species were brushed off the branch in the bag with a stiff brush. All invertebrates was then extracted from the bag with a suction sampling method, using an insect screen attached

to suction device (i.e. leaf blower). Invertebrates where stored in at -20˚C until identification and Stable Isotope Analysis. Species were identified in the lab to the lowest feasible level, mostly family. Likely food sources like fungi and bark were collected separately to determine stable isotope signature. All samples were dried at 60°C until stable weight and homogenized using a ball mill (Retch). Homogenized samples were weighed in tin cups and analyzed for carbon and nitrogen stable isotope composition on an isotope ratio mass spectrometer (IRMS, Thermo Scientific).

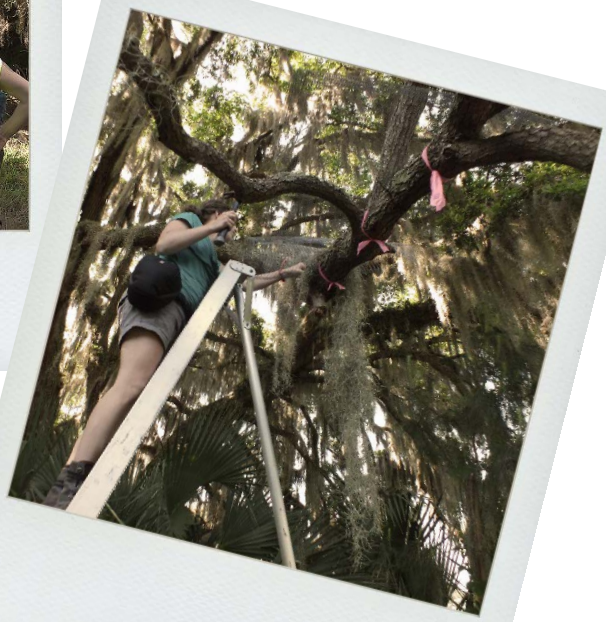
Intertidal Blue mussel beds at the wadden sea, The Netherlands

We sampled and reconstructed the food webs of 6 intertidal mussel bed and adjacent bare mudflat in a paired design across the Dutch Wadden Sea. All fauna was identified to species level. An extensive overview of the methods used to collect this food web is given in the paper of Christianen *et al.* (2017).

Water-starwort in Desselse Nete, Belgium

Aquatic macroinvertebrates were collected from 3 monospecific Water-starwort (*Callitriche obtusangula*) patches and neighbouring bare patches in the Desselse Nete, a sand bottom lowland stream in the north of Belgium (51°14'53" N, 5°4'53" E). In the studied reach, stream width varied between 3.5 and 5.5 m with an average depth of 58 cm and an average discharge was 0.69 m^3s^{-1}. Macroinvertebrates were collected both on the vegetation and in the sediment. Within macrophyte stands, macroinvertebrates were collected using a cylindrical box-sampler (total volume: 6663 cm^3). Macroinvertebrates in the sediment were collected inside each vegetation patch and in neighbouring bare patches. For each sediment sample three sediment cores were collected and pooled using a core sampler (diameter 5.4 cm), resulting in a total sediment area of 63.7 cm2 for each sample. Additional food web components, including organic matter, macrophytes and epiphyton, were also collected. Sestonic FPOM was filtered from river water over 55 µm Whatmann glass-fiber filters (GF/C), while CPOM was collected in both sestonic and benthic form, by sieving it from the river water and the upper 5 cm of the sediment, respectively. Aquatic macrophytes and epiphyton were both collected with the macroinvertebrate samples.

In the laboratory, the sediment and plant samples were sieved (mesh size 500 µm) and the macroinvertebrates were separated from the sediment and plant material. Macroinvertebrates were then stored at -20˚C until identification. Macroinvertebrates were identified to the lowest practical taxonomic level (generally species).



3. Food or furniture: separating trophic and non-trophic effects of Spanish moss (*Tillandsia usneoides*) to explain its high invertebrate diversity

Annieke C.W. Borst, Christine Angelini, Anne ten Berge, Leon Lamers, Marlous Derksen-Hooijberg, Tjisse van der Heide

Submitted

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Abstract

Foundation species are typically suggested to enhance the diversity of their associated community by increasing habitat structure and mitigating physical stress. In this classic perspective, the trophic role of foundation species in facilitating other species is considered of minor importance compared to their non-trophic role. Yet, studies that experimentally tease apart the relative importance of the trophic versus non-trophic effects of foundation species are rare. In a 3-month field experiment, we transplanted different patch sizes of living festoons and non-living, structural mimics of Tillandsia usneoides (Spanish moss), to assess the trophic and non-trophic effects of this well-known, widely distributed foundational epiphyte in mediating the arboreal invertebrate community. Compared to bare tree branches, living and mimic festoons enhanced invertebrate richness and abundance, clearly demonstrating not only a non-trophic, but also an additional trophic role of Spanish moss. Our results show that 40% of the total species richness increase in Spanish moss could be contributed to habitat structure alone, as simulated by plastic mimic festoons, while in addition, the trophic role of live Spanish moss further stimulated community species richness by another 60%. The enhancement of invertebrate species richness appeared to be independent of increasing patch size. Strikingly, we found that the trophic stimulation of the invertebrate community did not result from direct feeding on Spanish moss. Instead, the plants mostly fueled the invertebrate community through the provisioning of detritus from Spanish moss, as detritivores were specifically enhanced in the living festoons. Our results highlight that facilitation by foundation species can be driven by the combined effects of their non-trophic role as a physical structure, and their trophic role in the food web, and call for new studies that address the trophic role of foundation species via detritus.

Introduction

Foundation species are spatially dominant, habitat-forming organisms that enhance the richness and abundance of ecological communities (Bertness and Callaway 1994, Bruno et al. 2003). Trees, freshwater macrophytes, seagrasses, reef-forming bivalves and corals are all examples of such foundation species which create habitat for other species with their own body tissue (Jeppesen et al. 1992, Ellison et al. 2005, Coker et al. 2014, Christianen et al. 2016, van der Zee et al. 2016, Ali and Yan 2017). A major factor thought to underlie foundation species' enhancements of associated communities is their positive effect on habitat modification (Govenar 2010). Habitat structure is suggested to enhance species richness through a number of potentially co-dependent non-trophic mechanisms (Kovalenko et al. 2012). First, it can enhance niche availability by creating new microhabitats, temporally uncouple predator-prey interactions, and mitigate physical stress in harsh environments (Kovalenko et al. 2012, St Pierre and Kovalenko 2014). Secondly, habitat structure can also potentially increase productivity of secondary food sources, such as epiphyton or catching external detritus, that can further boost feeding guild richness and overall species richness (Kovalenko et al. 2012).

Next to these facilitative non-trophic pathways generated by increased habitat structure, foundation species may also increase biodiversity through its direct trophic effects as organic tissue, acting as a food source. Although most studies on foundation species and species richness contribute their facilitative effects to their habitat-modifying properties (Bertness and Callaway 1994, Bruno et al. 2003), only recently a number of studies have focused on their role in the food web (Miller et al. 2015, van der Zee et al. 2016). These studies suggested that the direct trophic role of foundation species as a food source is of minor importance, compared to their non-trophic habitat-structuring role. Yet, the majority of these studies were correlative or theoretical studies (Miller et al. 2015, van der Zee et al. 2016) or studied the contribution of secondary food sources concentrated by the foundation species (Verweij et al. 2006, Gartner et al. 2013) and only few studies have directly compared artificial and natural structures to experimentally separate trophic and non-trophic contribution of the foundation species itself on total community and feeding guild responses (Taniguchi et al. 2003). Also, it is unclear if the relative importance of these trophic or non-trophic pathways shifts with the patch size of foundation species (Angelini et al. 2015). Larger patches may sustain similar species densities (Li et al. 2017), but edge effects may change species dynamics and create non-linear community responses (Melo et al. 2016)

In this study, we experimentally tested the effects the trophic/non-trophic contributions provided by habitat-forming plants on associated species and feeding guild richness and the effect of patch size. More specifically, we tested the hypothesis that habitat-creating properties of the vascular epiphyte, Spanish moss (*Tillandsia usneoides,* hereafter Spanish moss) is a

stronger driver of the species richness, abundances and feeding guilds than its trophic role as a food source. Also, we hypothesize that, in line with earlier findings, invertebrate species number and abundance increase with patch size (Taniguchi et al. 2003, Matias et al. 2010, Gartner et al. 2013).

Spanish moss is a rootless bromeliad distributed from North Carolina US to central Brazil. It is common in the southeastern coastal plain of the United States where it prolifically and abundantly grows in the canopies of many trees including Southern live oaks (*Quercus virginia*, hereafter oak) and other trees (Garth 1964, Schlesinger and Marks 1977, Callaway et al. 2002). It grows in strands with alternating leaves that congregate in entangled clumps, called festoons, hanging from tree branches (Fig. 1). Through its festoon-forming structure, Spanish moss facilitates a wide range of invertebrate species – some of which exclusive to Spanish moss (Young and Lockley 1989) – that benefits from Spanish moss' mitigation of temperature and humidity stress and reduction of predation pressure (Angelini and Silliman 2014). Next to these non-trophic effects, Spanish moss may also serve a food source, as live plant tissue may attract herbivores and decaying plant tissue can serve as food for detritivores. As Spanish moss has a very low protein content with only 0.6% nitrogen content we expected that its non-trophic contribution (i.e. providing habitat structure, capturing particulate organic matter and mitigating stress) to species richness is more important, than its trophic contribution.

To test our hypothesis, we carried out a field experiment in which we compared bare branches, to branches draped with living Spanish moss festoons and with plastic mimics of Spanish moss that do not provide live plant tissues to support invertebrates but can trap aeolian particulate matter. We also manipulated the sizes of both plastic and living festoons to explore the effect of patch size. We compared species number, invertebrate abundance and feeding guilds as well as non-trophic effects between treatments. Our study reveals that trophic and non-trophic effects by foundation species can both have distinct effects on species richness and invertebrate abundances independent of patch size.

Methods

Study site

The study was conducted in the subtropical National Estuarine Research Reserve on Sapelo Island, Georgia, USA (31°24'49.1"N 81°17'19.4"W) from April to August 2014. The experiment was performed in savanna habitat dominated by live oaks (*Quercus virginia*) and Bahia grass (*Paspalum notatum*). The tree selected for the experiment was freestanding with its horizontal branches abundantly overgrown with Spanish moss (canopy diameter 28 meter). Spanish moss, a vascular CAM-photosynthesizing plant that forms large entangled festoons, was

chosen because of its easy manipulation, spatial dominance in the region, and its lack of a rhizosphere, leaving one sphere in which the species interactions take place.

Experimental design

To elucidate the effects of habitat structure and food availability we compared festoons of living Spanish moss (Fig. 1a) with artificial mimics of Spanish moss (Komodo products, Syston, UK) with similar complexity commercially produced for use in vivaria (Fig. 1b), which were rinsed thoroughly with water before use. Living Spanish moss festoons were collected, after which all invertebrates and dust were removed by vacuuming each festoon for 60 seconds with a suction sampler (i.e. a Stihl BG55 leaf blower with vacuum attachment fitted with insect netting). To test the effect of patch size, we established four festoon size classes for both living and mimic moss based on volume measured in a graduated cylinder. The smallest class was comparable in volume to small, newly grown festoons of Spanish moss and the largest size class was comparable to some of the largest festoons found in the oaks (Fig. 1a, Table S1, Angelini and Briggs 2015). Bare branches without any Spanish moss were used as a control.

Plots were set out on horizontal branches (branch diameter: 14 cm, sd: 7 cm) of the experimental oak between 1.5 meters and 3 meters off the ground and between 2 meters and 3 meters from the leaves. All Spanish moss within a 0.5 meter distance from each plot was removed and all plots were fitted with a mesh roofing to prevent falling Spanish moss fragments from entering the plots and altering treatments. Treatments were randomly assigned to the plots and the Spanish moss and mimics were strapped to the tree using a cable tie. The bare branch control plots were also fitted with a cable tie and a mesh roof.

Habitat complexity

Habitat complexity was interpreted in this study as the structural morphology of the plant. All festoons consisted of the same dichotomous strands typical for Spanish moss as the main complexity-generating element (Tokeshi and Arakaki 2012). By defining complexity this way it can be studied independently of patch size (Taniguchi et al. 2003, Tokeshi and Arakaki 2012). To test whether habitat complexity of the mimics was equal to that of living Spanish moss, we measured and compared the fractal dimensions and interstitial space of both. To this end, strands of Spanish moss and plastic mimics were photographed on a white background and converted to black and white images using Photoshop CS6. Next, the fractal dimensions were analyzed using the fractal box counting tool in ImageJ 1.51k (Rasband 1997). Another proxy for habitat complexity, interstitial space, was calculated according to the method of (Dibble et al. 1996) on the lowest festoon size classes. For this index vertical and horizontal axes were randomly drawn on scans of living and mimic festoons (n=3) (see examples in Fig.

S1) along which lengths and frequencies of interstices –gaps between stems and leaves- were measured, after which interstitial space (*I*) was calculated as follows:

$I_{hv} = (f_h/l_h) + (f_v/l_v)$

Where *f* is the mean frequency or the number of interstices and *I* Is the mean length (mm) of all interstices sampled along the horizontal (*h*) or vertical axes (*v*). A higher *I* value means a higher frequency and smaller gaps in the structure. The fractal dimensions and interstitial space of the plastic mimics (5.4 ± 1.06 and 1.16 ± 0.001, respectively) were statistically indistinguishable from living Spanish moss (8.5 ± 1.17 and 1.17 ± 0.003 respectively).

Temperature variation

Temperature was logged every 15 minutes for 4 days using iButton data loggers (Hygrochron, Embedded Data Systems, Lawrenceburg, Kentucky, USA) to measure the effect of Spanish moss and the mimics on their environment. Due to the limited number of available loggers, iButtons were only glued to branch surfaces in the bare branch, the extra-large Spanish moss and extra-large mimic treatments. Temperature mitigation was calculated by using the average overall measured temperature overall and calculating the absolute deviation from the average temperature on each time step.

Invertebrate sampling, nursery function, and dust collection

Three months after establishing the experiment, we enclosed each plot in a 190-L plastic bag, into which we placed the festoon (if present) and brushed the branch for 30 seconds to capture all present detritus and invertebrates. We collected all invertebrates and particulate matter by feeding the content of the bag over the suction sampler, which was fit with an insect screen bag. All macroinvertebrates were sorted and counted based on morphospecies (hereafter, species), feeding guild (i.e. predator, scavenger, detritivore, herbivore or parasite) and life stage (juvenile/adult). The function of Spanish moss as a nursery was quantified per festoon by counting the egg cases and cocoons in the suction samples and by scanning the festoons for remaining egg and cocoons.

Airborne particulate matter such as pollen and detritus may be an important food source to invertebrates. Hence, we measured the amount of particulate matter inside each festoon at the end of the experiment by collecting all the particles in an air filter behind the insect screen during the suction sampling. The dust sample was then transferred to a pre-weighted plastic bag and dried (24h at 60 °C), after which biomass was determined. Finally, the end volume of the festoons was measured again – three Spanish moss festoons (1 medium, 1 large and 1 extra-large) had lost volume due to shedding and were reclassified to be a smaller, appropriate size class.

Statistical analysis

All statistical analyses were done in R version 3.4.1. As a first step, we compared how extra-large living and mimic festoons compared to the bare branch treatment to test how the addition of structure alone versus structure and food affected the community responses: non-trophic effects of Spanish moss (i.e particulate matter and nursery events), species richness and evenness and feeding guild responses of the invertebrate community. We used generalized linear models with a Poisson distribution for count data and a Gaussian distribution for continuous data with One-Way ANOVA using the car package in R., followed by Tukey posthoc tests to detect differences between the 3 treatments (bare branch, extra-large living festoon, and extra-large mimic). In separate analyses in which the bare branches were not included, we then investigated the effect of festoon patch size. We used general linear models with festoon volume and festoon type (i.e. mimic or living) as factors. Continuous variables were checked for normality and log-transformed when necessary. Count data was analyzed using a Poisson distribution or negative binomial distribution when overdispersion was found. All models were then analyzed in a two-way ANOVA type 3. Temperature mitigation was analyzed using a linear mixed model (lmerTest in R) with treatment as fixed factor and time as a random factor.

Results

Non-trophic interactions of Spanish moss and mimics

Both living and mimic extra-large Spanish moss festoons stabilized temperatures relative to bare branches, where temperatures varied significantly more over the four days of iButton monitoring (Fig. 1c). Secondary food resources in the form of aeolian particulate matter increased 5.3 times in both living and mimic festoons relative to bare branches (Fig. 1d). Both festoon types acted as attachment substrate for egg cases and pupae (i.e. nursery events), increasing the amount of nursery events to 4 compared to the bare branch (Fig. 1e).

Festoons versus bare branch

The extra-large living and mimic festoons changed community metrics in various ways relative to bare branches, showing the strong potential for this foundation species to locally alter community structure and boost species richness. Species richness, expressed as the number of species, and guild richness (number of feeding guilds) were increased by the plastic mimics, but even more so by living festoons. Specifically, 6.7 times and 3.6 times respectively in mimic festoons compared to bare branch, while living festoons increased by 15.6 times and 6.7 times respectively (Fig. 2a, c). Evenness increased 12.7 times in both festoon types (Fig. 2b) and guild evenness was only significant for living Spanish moss, which increased 8.7 times compared to bare branch. Comparing the smallest festoons to bare branch also shows the same statistical

trends in biodiversity indicators, illustrating even smallest festoon is efficient to enhance species richness (Fig. S2)

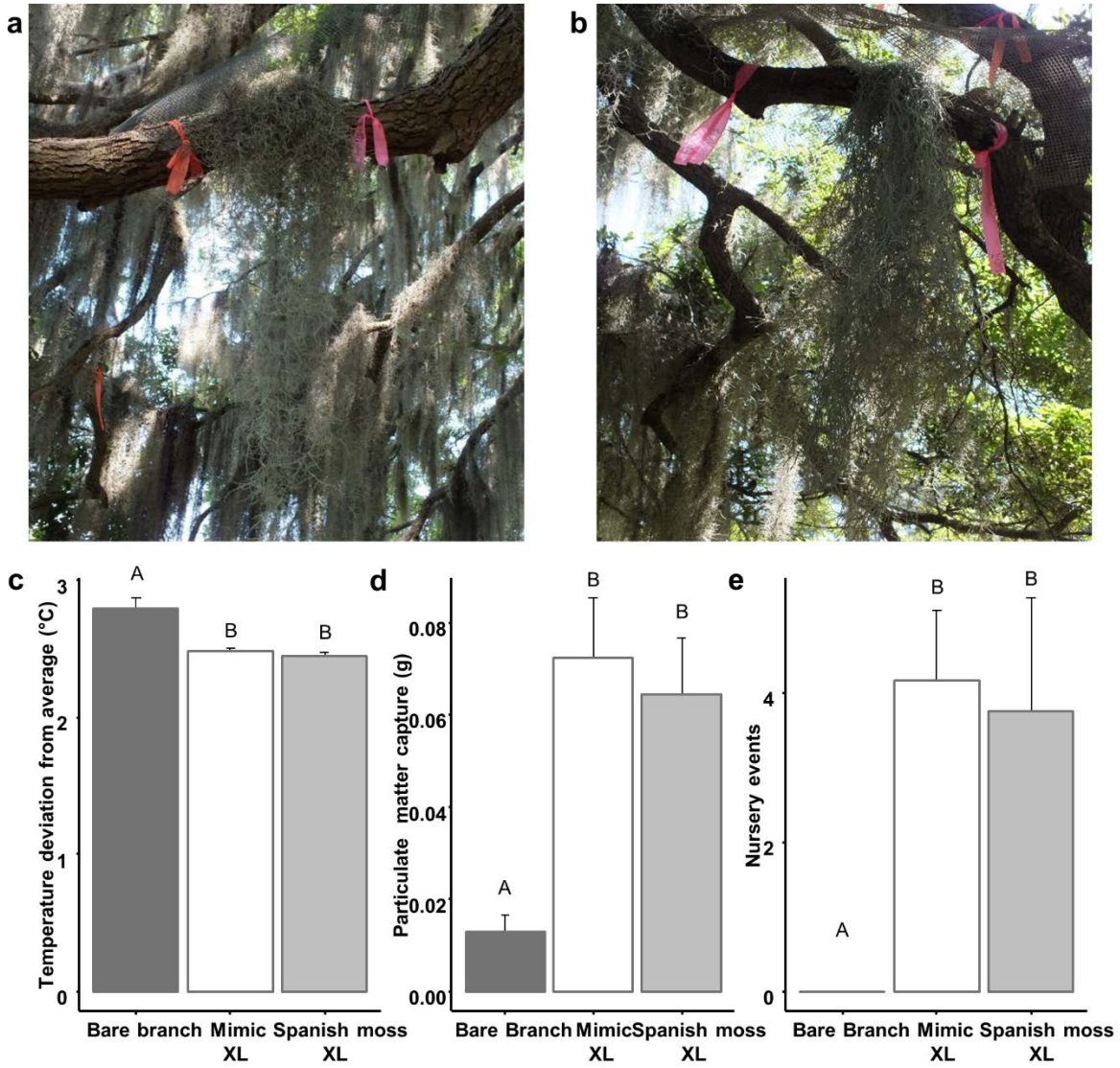

Figure 1. Potential non-trophic services measured in Spanish moss. a) The largest Spanish moss festoons and b) the largest mimic festoons compared to bare branch: c) temperature deviation, d) particulate matter capture and e) nursery function. Letters indicate posthoc grouping

Effects of festoon type and patch size

The number of nursery events increased with festoon patch size but did not differ between living and mimic festoons (Fig. 3a). The juvenile invertebrate counts also increased with festoon size, but was significantly lower in mimics compared to living festoons (Fig. 3b). We found significant differences between living and mimic festoons for species richness, evenness and guild evenness. Moreover, these were all dependent on festoon size, but without any interaction with festoon type (i.e. living or mimic) (Fig. 3c,d,f). Species richness increased 1.9 times from smallest to the largest size class in the mimic festoons, and 3 times in living Spanish

moss. Evenness increased 1.5 times in the mimics, and 1.6 times in Spanish moss (Fig. 3d). Guild richness was 1.8 times higher overall in Spanish moss compared to mimics. Finally, guild evenness did show a dependency on festoon size and type; mimics increased 1.6 times from smallest to largest while living Spanish moss increased 1.4 times (Fig. 3e).

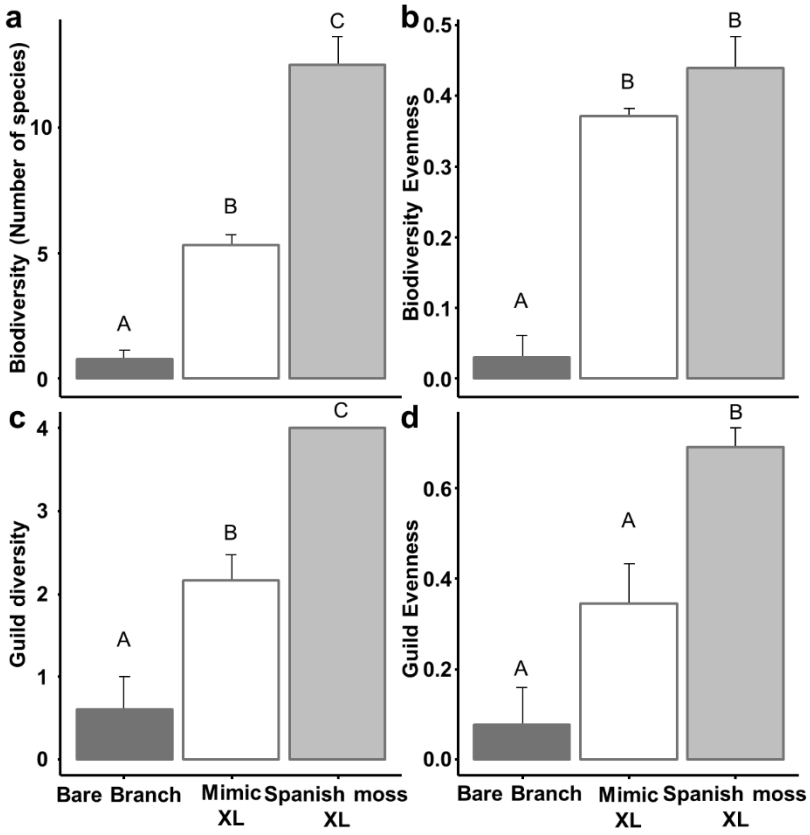

Figure 2. Biodiversity indicators for the largest festoons of Spanish moss (light grey) and plastic mimics (white), with bare branch (dark grey) as a control. Letters indicate posthoc grouping. a) Species richness expressed as the number of species, b) Species Evenness, c) guild richness, expressed as the number of guilds, d) guild evenness.

Herbivores did not depend on festoon size or type and were less abundant than other feeding guilds (Fig. 4a). In contrast, detritivores, scavengers and predators were all significantly affected by festoon size and festoon type (Fig.4b-d). Detritivores, which were 99% isopods, were rare in mimic festoons and only increased slightly in abundance with increasing festoon size (i.e. from 0 to 2 individuals per festoon in small versus extra-large festoons), in contrast to the living festoons increased 21.3 times in Spanish moss. Scavengers, which were mostly cockroaches and common scaly crickets, increased from 0 to 1.5 in mimics from small to extra-large and from 0.7 to 7 in living Spanish moss. Predators, 98% of which were spiders, increased 2.7 times in mimics and 7 times in living Spanish moss.

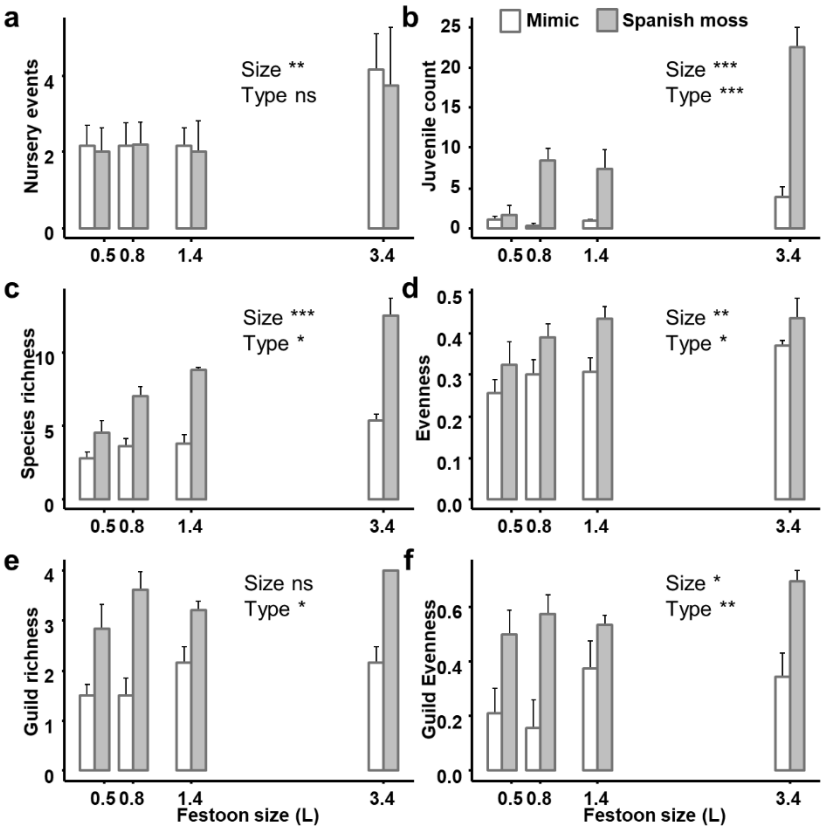

Figure 3. Biodiversity indicators (mean ± SE) for all festoon patch sizes and both festoon types (living or mimic) with statistics results, no interactive effects were found. a) Number of nursery events scored per festoon b) juvenile macroinvertebrates scored per festoon c) Species richness as the number of species, d) evenness of species, e) guild richness expressed as the number of guilds present and f) evenness of guilds.

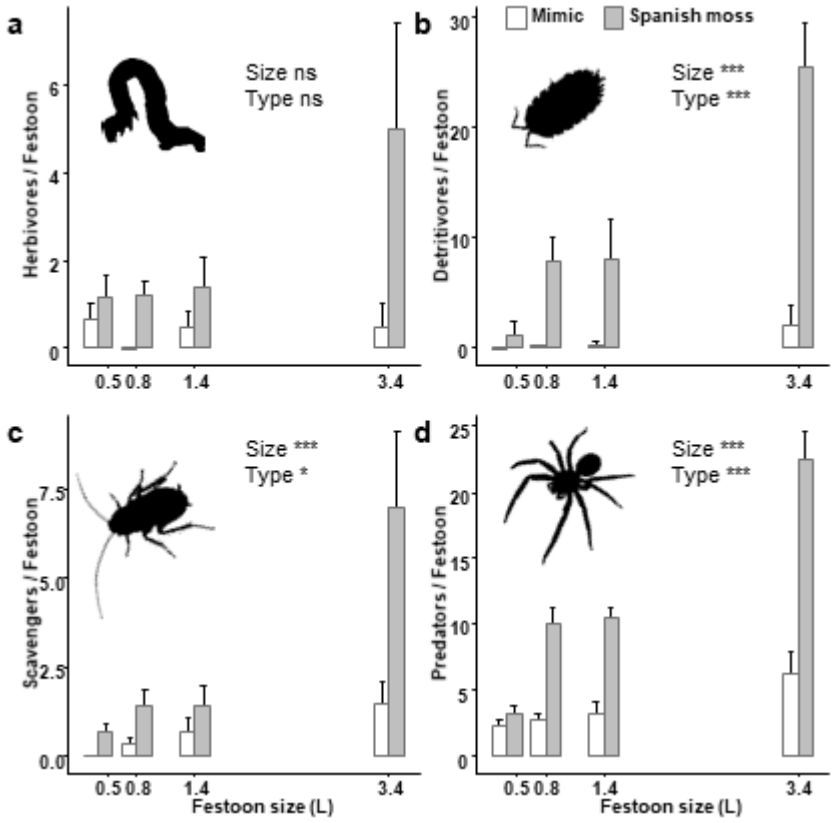

Figure 4. *Invertebrate counts of different feeding guilds for all festoon patch sizes and both festoon types (living or mimic) with statistics results, no interactive effects were found. (bars represent mean ± SE). a) Herbivores, b) detritivores, c) scavengers, d) predators*

Discussion

Our results show that the non-trophic role of Spanish moss festoons, as well as their role as a food source, both strongly increased the species richness and abundance of the invertebrate community with increasing patch size. Moreover, we found that trophic/non-trophic contributions (living vs mimic) stimulated community richness seemingly acted independently from patch size, since we did not identify any interactions. We found that non-trophic facilitation (structure) alone, as simulated by plastic mimic festoons, increased biodiversity indicators by 3.6 to 12.7 times compared to bare branch controls. Furthermore, in contrast to our hypothesis, species richness was stimulated much more within the living Spanish moss festoons. Specifically, our results demonstrate that, when added to the effect of habitat structure, the trophic role of Spanish moss increases biodiversity indicators by 6.7 to 15.6 times. We therefore conclude that the total species richness depends for about 40% on habitat structure and, on top of that, 60% depends on food provisioning by the festoons themselves. These results highlight that facilitation by foundation species can be driven by the combined effects of their non-trophic, habitat-structuring role, and their trophic role in the food web, and that these effects are independent of patch size.

Non-trophic, habitat-structuring, effects of Spanish moss

We demonstrated that by generating habitat structure, Spanish moss strongly changes ecosystem functions and species richness. The results show that both the mimic and living festoons similarly mitigated temperature fluctuations, trapped similar amounts of particulate matter, and were also indistinguishable in their role as a nursery. The addition of habitat alone by the mimic festoons resulted in a dramatic enhancement of species richness compared to bare branch. Moreover, even the smallest mimic festoons clearly stimulated species richness and community evenness compared to bare branches. Previous work from Angelini et al. (2014) suggests that together with the live oak on which it grows, Spanish moss forms a facilitation cascade, in which the moss acts as a secondary foundation species. In general, foundation species are suggested to enhance species richness by increasing habitat structure, and mitigating physical stress (Bertness et al. 1999, Govenar 2010, van der Zee et al. 2015, van der Zee et al. 2016). Although our results show that Spanish moss indeed reduces temperature fluctuations, we found this mitigating effect to be relatively minor – i.e. less than 0.5^{o}C reduction in deviation from the average compared to bare branch. Hence, we suggest that in our case, enhancement of habitat structure was likely more important in shaping the Spanish moss invertebrate community. Although the mechanism driving such a biodiversity-habitat complexity relationship are still not completely understood (Kovalenko et al. 2012), enhancement of niche availability through the creation of new (micro-)habitat and alterations in predator-prey interactions have been suggested as important factors (Bertness et al. 1999, Kovalenko et al. 2012, St Pierre and Kovalenko 2014). Additionally, habitat structure may also stimulate the community indirectly, by acting as a resource concentration mechanism (Kovalenko *et al.* 2012).

Indirect resource concentration

Apart from offering increased habitat structure, and mitigating physical stress, we found that both the mimic and living Spanish moss festoons increasingly trapped particulate matter with increasing festoon size (Fig. S3). This suggest that virtually all of this accumulated matter originated from outside of the festoons. By trapping external resources Spanish moss festoons can indirectly fuel the food web through the indirect provisioning of resources. Earlier work on the relationship between such secondary food sources and habitat structure yielded varying results (Taniguchi et al. 2003, Ferreiro et al. 2011, Verdonschot et al. 2012, Loke and Todd 2016). Patch size of plants has been positively linked with accumulation of external food sources (Taniguchi et al. 2003). Nevertheless, the extent to which these food sources drive biodiversity across ecosystems has yet to be disentangled from the other non-trophic effects of habitat structure. While, as an indirect trophic effect of foundation species, they may serve an important role for part of the food web (e.g. see Fig. S3 Chapter 2).

Trophic effects of Spanish moss

Compared to the mimics, living festoons clearly had a stronger facilitating effect on the community, which most likely resulted from the trophic role of the plants themselves, as we did not detect any differences in non-trophic effects (i.e. habitat complexity indices, temperature mitigation, particulate matter capture and nursery function) between mimic and living festoons. Although Spanish moss thus appears to also have a direct trophic role next to its habitat-structuring (including resource-concentrating) role, the particular enhancement of the detritivores suggest that it serves primarily as an important food source in the form of detritus. Detritivores and scavengers were dramatically (4 and 15 times respectively) increased in living Spanish moss compared to the mimics, whereas herbivores were unaffected and numbers were low compared to the other guilds. This stimulation of the 'brown food web' – i.e. food webs with detritus as the dominant carbon input rather than living plant matter that drive green food webs – indirectly also appears to facilitate predators, which were also much more dominant within living Spanish moss. As predators were dominated by spiders in our study system, and web-weaving spiders in particular, we would expect that habitat structure would be of particular importance to this group (diminishing the effect of living vs mimic plants). In contrast, however, this group was greatly enhanced in living Spanish moss compared to the mimics (Fig. S4) without any interaction with festoon size. These results thus suggest that most predators in the festoons actually depend on local rather than external prey – probably mostly detritivores and scavengers – even in the case of web weaving spiders.

Trophic versus non-trophic effects

Recent empirical, but correlative, studies investigating the non-trophic and trophic roles of foundation species suggest that their non-trophic interactions are far more important than trophic interactions in facilitating other species (Miller et al. 2015, Christianen et al. 2016, van der Zee et al. 2016). These studies argue that foundation species are typically rather unpalatable and are therefore relatively unimportant as a food source. Although our experimental manipulations indeed support the notion that Spanish moss, as a secondary foundation species (Angelini and Silliman 2014; Chapter 2), is relatively unimportant as living plant tissue, they also show that it has an important trophic role by stimulating the brown food web via decaying plant tissue. Although thus far hardly considered, such stimuli of the food web by foundation species may also be important in many other foundation species-structured ecosystems as well. For instance, studies on kelp trying to disentangle the contribution of detritus of kelp and phytoplankton, seem to show a substantial contribution of kelp detritus to the diet of suspension feeders (Kaehler et al. 2000, Kaehler et al. 2006). This view is supported by earlier work showing that detritus is often pooled as one homogeneous food resource, rather than a separate compartment in food web studies, and emphasize the urgent need to

disentangle trophic pathways mediated by the brown web (Moore et al. 2004, Miller and Page 2012, Campanya-Llovet et al. 2017). In this study, we provide compelling evidence that foundation species, next to their structuring role, can significantly contribute as a food source via the brown food web.

Conclusion

Overall, we conclude that foundation species can stimulate community-level biodiversity through multiple distinct pathways. First of all, our experimental results confirm the notion that foundation species, by increasing habitat structure, enhance both species and guild richness. Importantly, however, our results also provide unequivocal evidence that when their detritus is processed locally in the brown part of the food web, foundation species can greatly stimulate biodiversity. Moreover, we show that the effects of habitat structure and food availability may act independently of patch size to increase biodiversity.

Acknowledgements

The authors would like to thank Peter Cruijsen and Jasper Hoogveld for their help in the field.

Supplementary Materials

Table S1. Average volume per size class of Spanish moss and mimics.

Treatment	Festoon size (Liters)		Average Festoon size
	Living Spanish moss	Plastic mimics	
Small	0.5± 0.05 (6)	0.5 ± 0.05 (6)	0.5
Medium	0.8 ± 0.15 (5)	0.8 ± 0.13 (6)	0.8
Large	1.3 ± 0.13 (5)	1.4 ± 0.08 (6)	1.4
Extra-large	3.2 ± 0.38 (4)	3.5 ± 0.2 (6)	3.4
Bare Branch	0 ± 0 (5)		0

Figure S1. Sample picture of interstitial space measurements.

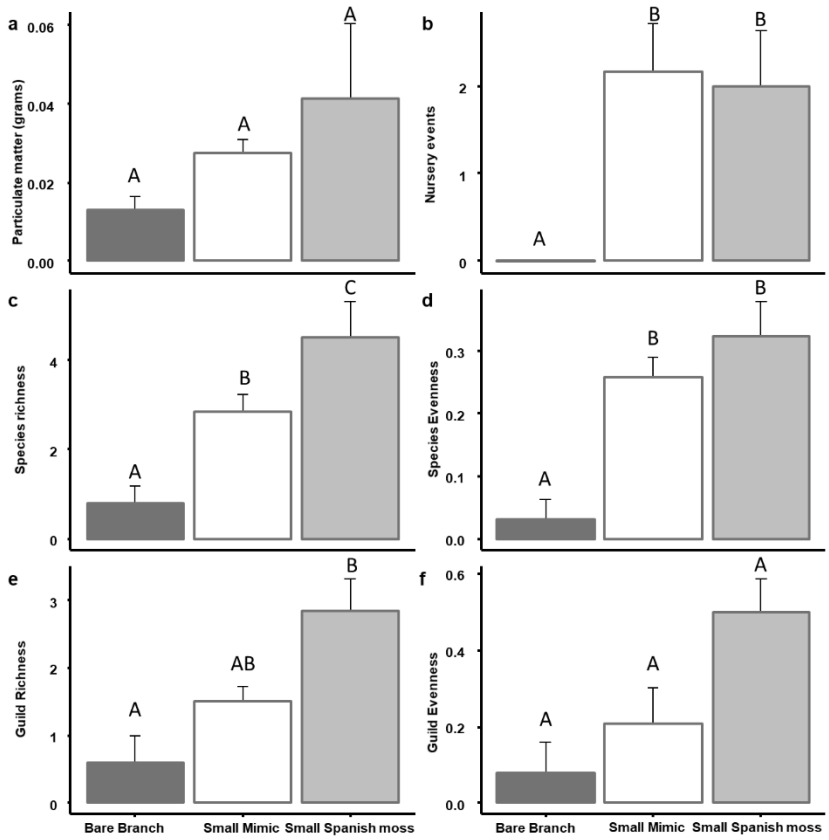

Figure S2 *Particulate matter captured, nursery function and biodiversity indicators per small festoon of Spanish moss (light grey) and mimics (white), with bare branch (dark grey) as a control. Letters indicate Posthoc grouping. a) Particulate matter in grams per plot, b) nursery events as the number of egg and cocoon cases. c) Species richness as the number of species d) species evenness e) guild richness as number of guilds f) guild evenness.*

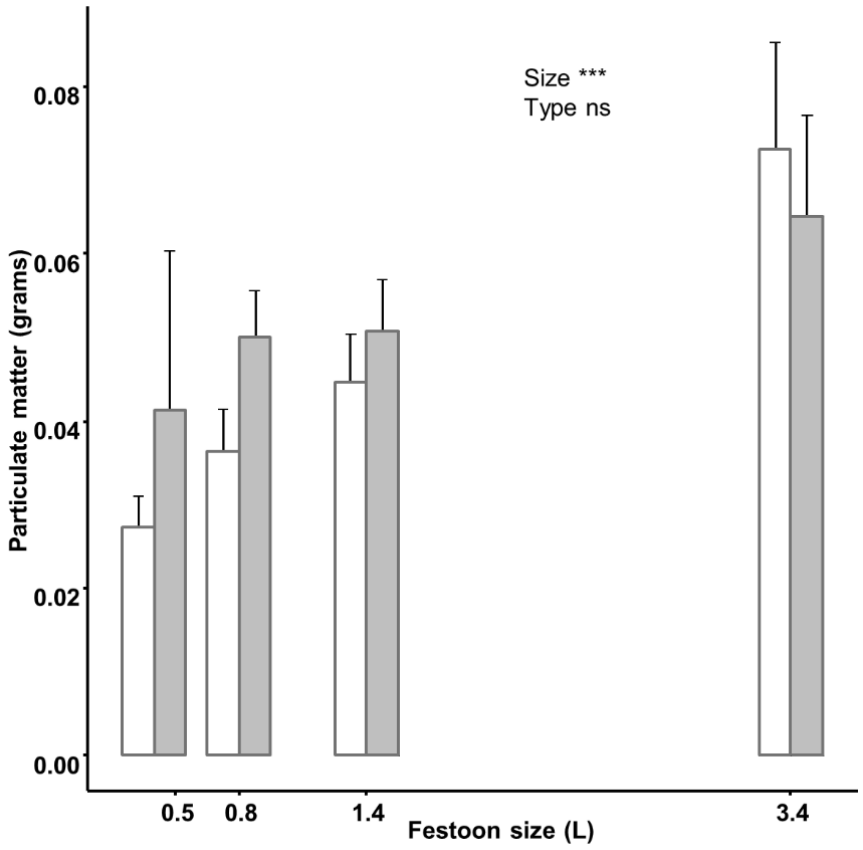

Figure S3. Particulate matter capture per size class for mimic (white) and living (grey) festoons

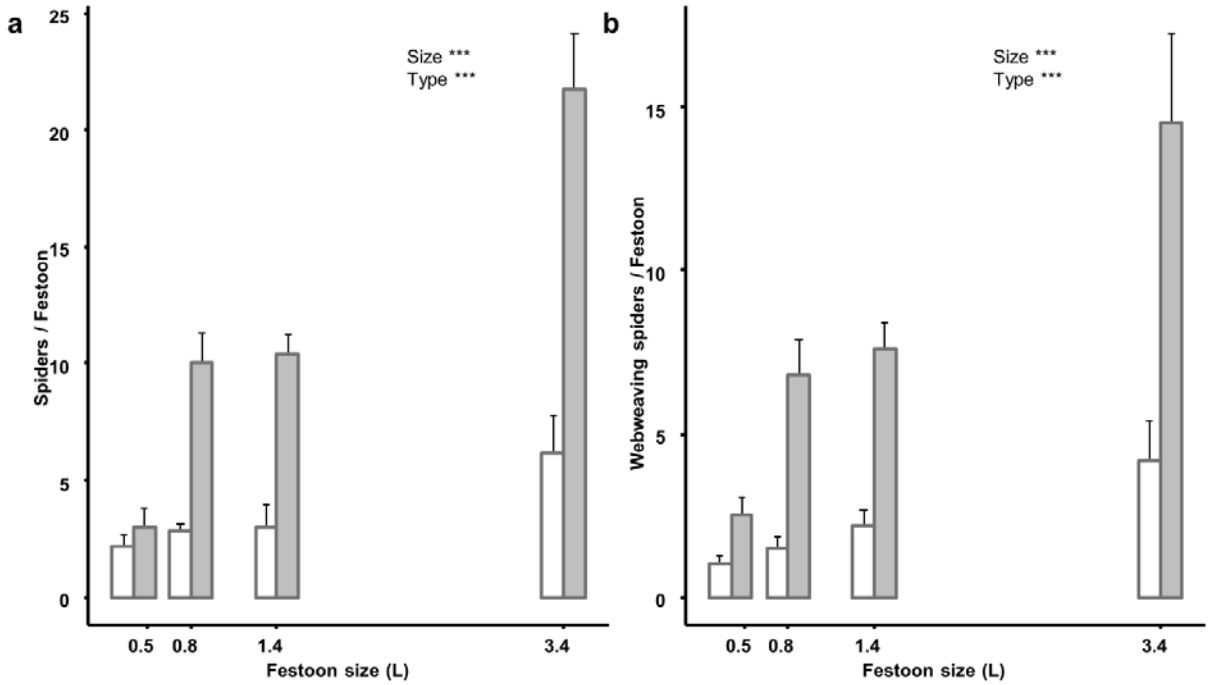

Figure S4. Spider counts and web weaving spider count per festoon size treatment in mimic (white) and living Spanish moss (grey).

4. Epiphyte-tree mutualism: Spanish moss significantly increases nitrogen availability to host oak trees

Eva van den Elzen, Annieke C.W. Borst, Christine Angelini, Hallie Fischman, Tjisse van der Heide, Leon P.M. Lamers

Vervang door opgemaakte versie

Abstract

Epiphyte-host interactions are generally presumed to be commensalistic (neutral effect on host) or parasitic (negative effect on host). Here we show that this relationship can also be mutualistic, when epiphytes enhance the availability of nutrients to their host. Tillandsia usneoides (Spanish moss) is a common vascular epiphyte in neotropical and subtropical regions of the Americas that benefits from the substrate and physico-climatological conditions generated by their tree hosts. However, it is unknown whether Tillandsia's effect on its host tree can be positive. We here investigate whether Tillandsia festoons significantly contribute to the nitrogen (N) budget of their host tree, Quercus virginiana (Southern live oak), and study how Tillandsia acquires its N. We found that the presence of decomposing Tillandsia festoons doubled N availability in the topsoil under the tree, where tree fine roots are concentrated. Data on isotopic composition revealed that Tillandsia obtained its N almost exclusively from atmospheric deposition instead of leachates from the tree. The main N input appeared to be diffusive uptake of NH_x (ammonia gas and ammonium). N captured by dust and debris (3.4%) or atmospheric N_2 fixation by Tillandsia's microbiome (<1%) did not significantly contribute to the total N budget of Tillandsia. Our findings show that Tillandsia festoons function as an atmospheric N filter, efficiently assimilating inorganic N from dry and wet deposition, and releasing organic N only after decomposition on the forest floor. In this way, Tillandsia provides the oak with an ecologically significant 5-24% (representing 0.6 to 3.6 kg N ha^{-1} y^{-1}) of its total aboveground N input including leaf recycling,. Given that N availability limits oak growth, our results show a mutualistic relationship between tree and epiphyte.

Introduction

Symbiotic relations between epiphytes, representing 10% of the world's plant species (Nieder *et al.* 2001), and trees are keystone associations in a range of ecosystems, including tropical rainforests, montane cloud forests, and tropical and subtropical swamps and savannas (Benzing 1990; Zotz & Hietz 2001; Scarano 2002; Francisco *et al.* 2018). Epiphytic communities are ecologically important as they increase both floral (Zotz & Hietz 2001) and macrofaunal biodiversity through their role as a structurally complex secondary foundation species (Young & Lockley 1989; Angelini & Silliman 2014; Angelini & Briggs 2015). The host trees act as a primary foundation species, providing an optimum habitat for epiphytes through structure and stable physico-climatological environment (Zotz & Hietz 2001; Ellison *et al.* 2005; Angelini & Briggs 2015). Epiphyte-host interactions are generally presumed to be either commensalistic, whereby the tree provides optimal habitat for the epiphyte and the epiphyte has little effect on its host (Blick & Burns 2009), or parasitic, whereby the epiphyte not only benefits from but also harms the host, based on structural or direct competition for the same resources (Benzing & Seemann 1978; Flores-Palacios 2016). Lacking access to the soil, holo-epiphytes can obtain their nutrients from atmospheric sources, directly from the host's vascular tissue, or indirectly from their host via leachates (Benzing & Seemann 1978; Press & Phoenix 2005). When atmospherically recruited and recycled, epiphytic nutrient capital likely plays an important role in nutrient conservation in the ecosystem (Coxson & Nadkarni 1995; Zotz & Hietz 2001). This means that the nature of the symbiosis between epiphytes and host trees may be largely determined by the epiphyte's means of nutrient assimilation and cycling. Little is known, however, about epiphytes' main sources of nutrients and their potential impact on the nutrient budget of their host trees.

The epiphyte nutrient pool can vary substantially between different forest types due to variation in epiphyte biomass and the total aboveground nutrient pool of the ecosystem (Nadkarni 1984). In tropical forests, for example, where epiphyte biomass represents only a small percentage (< 5%) of the biomass of their host tree, their nutrient levels can represent up to 45% of total nutrients contained in the tree's foliage (Nadkarni 1984). In contrast, epiphytes may represent a much larger proportion of biomass in savannas, the most common ecosystem in the tropics and subtropics (Scholes & Archer 1997). Savannas are open grassland systems with scattered trees in which productivity is generally limited by nitrogen (N) availability (Tilman 1986; Bustamante *et al.* 2006). In coastal savannas located in the southeastern US and central and South America, oaks form broad, sprawling canopies that can be covered by a conspicuous, dominant vascular epiphyte, *Tillandsia usneoides* (hereafter *Tillandsia* (Garth 1964; Angelini & Silliman 2014). This epiphyte, also called Spanish moss, is the most broadly distributed bromeliad and is well known in the USA, where it defines the

landscape aesthetic (Garth 1964; Benzing 1990). *Tillandsia* prefers *Quercus virginiana*, southern live oak (hereafter oak) as its host tree, but is also found in pines, loblollies, and non-live structures like electricity poles, suggesting some degree of structural and nutrient independence from the host tree (Abril & Bucher 2009).

Tillandsia does not absorb nutrients through its rudimentary roots, but via its layer of absorbing scales on the leaves (Garth 1964; Benzing 1990). Tillandsia can therefor only obtain its nutrients in four ways: 1) indirectly from their tree host via leaching or decomposition of host tissue, 2) directly from the atmosphere via wet deposition and dry deposition (aerosols), 3) from airborne dust and excreta of fauna associated with the canopy, or 4) through associations with symbionts such as N_2-fixing microorganisms. The first way would suggest an interaction, in which *Tillandsia* intercepts nutrients from the tree (Benzing & Seemann 1978). However, direct uptake of airborne nutrients (2 and 3) is expected to be more important, given that these epiphytes developed trichomes, i.e. specialized epidermal outgrowths which increase the plant's surface area, creating a kind of brush border for trapping debris and taking up airborne water and nutrients (Angelini & Silliman 2014). In addition, *Tillandsia* functions as a secondary foundation species, improving conditions in the host tree for a biodiverse faunal community (Angelini & Silliman 2014). Excreta from this community together with other dust caught between the trichomes of *Tillandsia* leaves potentially form another nutrient input (Benzing 1981; Nadkarni 1986; Van Stan *et al.* 2015). Lastly, trichomes form a favorable habitat for a diverse community of microorganisms that contribute to plant and ecosystem functioning (Lindow & Brandl 2003), including the potential input of N by N_2 fixation (Brighigna *et al.* 1992), as is known for mosses and lichens (DeLuca *et al.* 2002; Cornelissen *et al.* 2007; van den Elzen *et al.* 2017) (4). As N_2 fixation is typically stimulated by more humid conditions, precipitation may stimulate the exchange of nutrients between *Tillandsia* and its associated microorganisms. Nutrient uptake via atmospheric deposition, dust and N_2 fixation are ways of nutrient collection that add to the available nutrient budget without taking up nutrients that may otherwise be used by the host tree.

When *Tillandsia* festoons dislodge and land on the ground, they are rapidly decomposed by invertebrates and fungi, increasing litter layer depth and altering the understory invertebrate and fungal communities (Angelini & Briggs 2015). Through this decomposition process, *Tillandsia* could function as a slow-release fertilizer for the host tree and supplying the host tree with an additional source of nutrients (Lang *et al.* 1976; Nadkarni 1984; Van Stan *et al.* 2015). Increasing *Tillandsia* density in the tree canopy was found to correlate linearly with increased *Tillandsia* deposition on the forest floor (Angelini & Briggs 2015), which may indicate that trees supporting high *Tillandsia* densities may be especially likely to benefit from epiphyte-mediated nutrient load enhancement. On the one hand, when *Tillandsia* gets its nutrients primarily from leachates and fallthrough, *Tillandsia* festoons may also be taking up

large amounts of nutrients, which then are not directly available to the tree host (Benzing & Seemann 1978; Umana & Wanek 2010). Thus, the nature of the symbiosis between host tree and epiphyte is unknown, and may be mutualistic, as the tree provides optimal habitat to the epiphyte while the epiphyte may simultaneously provide additional nutrients to the host tree.

Here, we identify the processes through which *Tillandsia* acquires its N, determine the role *Tillandsia* decomposition plays in contributing N to the soil, and resolve the nature of the symbiosis between *Tillandsia* and southern live oaks. First, we aim to unravel how *Tillandsia* obtains its N by studying the contribution of N_2 fixation of its microbial symbionts by isotope labeling, and of dust and captured rainwater using their isotopic composition. Second, we quantified the N concentration in the soil beneath festoons and compared it to uncovered soil to investigate how dislodged *Tillandsia* affects N availability in the soil. Last, we quantified the total nutrient load of *Tillandsia* available for the tree at different densities to determine if *Tillandsia* contributes to the total N input of the tree or if it takes away from the trees N supply. We hypothesized 1) that, of the four types of nutrient uptake, N_2 fixation by microbial symbionts is the main N source for *Tillandsia*, as it is a well-known pathway for other rootless plants and lichens (Turetsky 2003; Lindo *et al.* 2013). In addition, we hypothesized 2) that this abundantly growing epiphyte represents an important addition to the nutrient input of *Q. Virginia* through the decomposition of additionally captured N, making their interaction mutualistic.

Methods

Our study was conducted in the National Estuarine Research Reserve on Sapelo Island, Georgia, USA (31°24'2"N, 81°17'4"W), a Pleistocene barrier island of approximately 7000 ha with a subtropical climate characterized by hot, humid summers and mild winters. The southern live oak, *Quercus virginiana* (Duncan & Duncan 1988) is the most common tree species in expansive, open savanna habitats, where their broad crowns cover between 5-65% of the area dominated by Bahia grass (*Paspalum notatum*). Similar to other locations in the southeastern US coastal plains, these evergreen oaks are loaded with the abundant vascular epiphyte Spanish moss, *Tillandsia usneoides* (Fig. 1) (Callaway *et al.* 2002). This representative of the Bromeliaceae family is distributed in festoons hanging from branches of the tree in variable densities. We selected seven trees with a crown diameter of 18-25 m, distributed at least 200m apart and with a volume of *Tillandsia* festoons of around 800 m^3 per tree (see methods of (Angelini & Briggs 2015)).

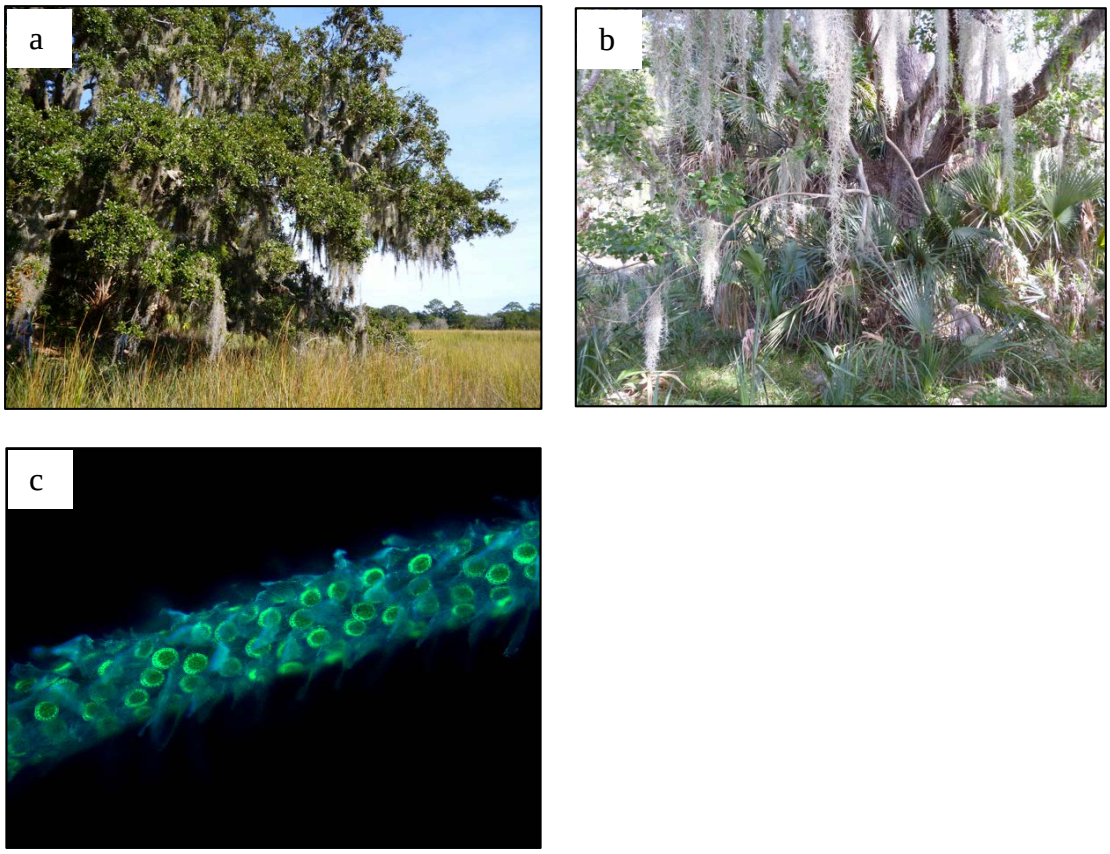

Figure 1. Pictures of study species: a) Quercus virginiana host tree in Savanna habitat. b) Tillandsia usneoides in host tree. c) fluorescence microscopic picture of DAPI-colored trichome structures on Tillandsia tissue.

Mineralization experiment

To find out how much N was mineralized from decomposing *Tillandsia* biomass, we conducted a decomposition experiment. Under each oak tree (N=7) soil and litter was collected in October 2013. Litter samples were taken from a surface of 10x10 cm and 5 of these subsamples per tree were mixed, homogenized and weighted. From these squares soil samples were taken with a plastic corer (ø=2.8cm, length 10 cm) and the depth of the organic humus layer was noted. Again 5 subsamples were homogenized, humus and topsoil layer separately, and weighted. Furthermore, from 6 of the 7 trees additional soil samples (2 subsamples per tree) were taken from underneath large *Tillandsia* festoons (of around 50-60 g dry weight (DW), that were experimentally placed and labeled to measure decomposition rates (Angelini & Briggs 2015). These samples were also divided into humus and topsoil samples, homogenized and weighted. Additionally, 5 living leaves from different branches of each tree were collected and pooled for nutrient analysis. All soil and leaf samples were chilled and transported to the lab in Nijmegen, the Netherlands. In the lab, plant-available N, including NH_4^+ attached to soil cation exchange sites, was estimated for all humus and topsoil samples, by shaking 17.5 g fresh weight (FW) in

50 ml of 0.2 M NaCl solution for 1 hour at 105 rpm (De Graaf *et al.* 2009). NO_3 and NH_4 concentrations were measured colorimetrically with an Auto Analyzer system (Bran and Luebbe, Norderstedt, Germany) using hydrazine sulfate (Kamphake *et al.* 1967) and salicylate (Grasshoff & Johannsen 1972).

N_2 fixation by Tillandsia's microbiome

To assess microbial N_2 fixation rates, we selected 3 large *Tillandsia* festoons each from 6 oak trees in October 2013. Each festoon weighed around 40 g DW, had a length of around 1 meter length, and hung halfway between the trunk and the canopy edge of the tree, at 1-3 meters above the ground. From each festoon, 2 samples were taken from the lower part (20 cm from lower end) and upper part (50-70 cm from lower end). Festoon samples were pooled per tree and put in 60ml glass vials for N_2 fixation assays. To half of the vials 3 ml of demineralized water was added to study whether increased humidity had an effect on N_2 fixation. Vials were capped and 45ml of the headspace was removed with an injection needle and replaced with $^{15}N_2$ gas (98atom % ^{15}N, Sigma-Aldrich, Germany), leading to a 75% $^{15}N_2$ labeling. Samples were incubated for 48 hours under one of the oak trees at 1m height. For each incubated sample, a control sample was collected to correct for the natural background abundance of ^{15}N. After incubation, enriched and control samples were dried and transported to the lab in Nijmegen, the Netherlands. Here, samples were ground using a mixer mill (MM301, Retsch, Germany) for 2 min at 30 rotations s^{-1} and total N concentrations and isotopic ratios were determined using an elemental analyzer (Type NA 1500 Carlo Erba, Thermo Fisher Scientific Inc., USA) coupled online via an interface (Finnigan Conflo III) to a mass spectrometer (Thermo Finnigan DeltaPlus, USA). For each sample the enriched isotopic N value was diminished with the average background isotopic N value of the control samples. These increases in ^{15}N labeling were converted to N_2 fixation rates (nmol N_2 gDW^{-1} d^{-1}) using the incubation time and weight of the sample.

Nutrient concentrations in leaves and Tillandsia

After being dried and ground by a mixer mill, total C and N in *Tillandsia* and oak leaves was assessed using an elemental analyzer (see above). Potassium (K) and phosphorus (P) concentrations of leaves and *Tillandsia* were determined by digesting 200 mg of sample in 500 µL HNO_3 (65%) and 200 µL H_2O_2 (30%) by heating for 16 min in a microwave (m.l.s. 1200 Mega, Milestone Inc., Sorisole, Italy). Digestates were diluted in demineralized water and P and K concentrations were measured by inductively coupled plasma emission spectrometry (IRIS Intrepid II, Thermo Electron corporation, Franklin, MA, USA).

Collection of Tillandsia dust

From one of the 6 selected oak trees, additional data on particulate matter associated with *Tillandsia* festoons was gathered in April 2014. Five large *Tillandsia* festoons hanging between 1.5-3 m from the ground were selected and dust present on the tissue was extracted using a suction sampler, i.e. a reversed leaf blower contraption. The festoons were then put back into the tree and all surrounding *Tillandsia* festoons within 0.5m were removed. After 3 months, the festoons were recollected and suction sampled again using a filter mesh of 0.5 mm and another of 0.2 mm, resulting in a sample of dust particles (0.2-0.5mm) for each festoon. These samples were dried and transported to the lab in Nijmegen, the Netherlands. The N concentration and isotopic signature were determined as described above. Bark and leaves from this tree were also collected for stable isotope analysis.

Wet and dry deposition collection

To compare the composition of wet deposition under *Tillandsia* festoons and bare branches, throughfall rainwater was collected in July-August 2017. All 7 oak trees were included with 6 rain collectors each: 3 under *Tillandsia* and 3 under bare branches. Rain was collected within 48 hours after 3 subsequent rain events of more than 0.2 mm of rain, after at least 2 days of drought, spread over 16 days. After each event total volumes of rain in collectors were taken and a pooled sample of 3 replicates per tree was stored in a fridge. All samples were transported to the lab in the Netherlands and concentrations of NO_3 and NH_4 were determined as described above. Total volumes and N amounts (NO_3 and NH_4 combined) in rain of the three events were summed up.

Statistics

N_2 fixation rates were tested with ANOVA, treating festoon part (upper vs lower) and humidity (dry vs. moist) during incubation as independent factors. Differences in total volume and N-load (i.e. mg N per m^2) in wet deposition between bare branches and *Tillandsia* covered branches were tested with a one-way ANOVA. The NH_4 and NO_3 availabilities in humus and topsoil were tested with an ANOVA with presence/ absence of *Tillandsia* on soil as independent factor. In all tests, residuals were normally distributed, confirmed by the Shapiro Wilk test, and the homogeneity of variance assumption was upheld based on Levene's test.. All analyses were carried out using IBM SPSS Statistics 21.0.

Results

N budget of Tillandsia

Tillandsia consisted of only 0.6% N (Table 1), which was much lower than oak leaves (2.0 % N). Relative contributions of different N sources were calculated based on a relative growth (biomass increase) of 45% per year (Angelini & Briggs 2015)resulting in an average total assimilation rate of 2.4 mg N g DW^{-1} y-1 (Table 2). N_2 fixation rates were found to be around 6.6 µg N_2 g DW^{-1} y^{-1}, representing only 0.3% of the total N input of *Tillandsia* (Table 2). No significant differences in N_2 fixation rates were found between different parts of large *Tillandsia* festoons, i.e. the lowest tip compared to the upper part ($F_{3,20}$=0.012; ns). Moreover, equal rates were found for incubations of *Tillandsia* under moister or drier conditions ($F_{3,20}$=0.110; ns). As a second N source, the capture of dust in the form of faunal excreta and dust particles was quantified. We found that in 3 months 45-70 mg of dust was caught by large festoons of around 41 g DW. This translates to 4.4-6.8 mg dust g DW^{-1} y^{-1}, of which around 1.5% is N, providing 81.8 µg N gDW^{-1} y^{-1} to *Tillandsia* and equaling 3.4% of its total N input (Table 2). This indicates that N_2 fixation and capturing of dust represent only small sources of N and that the majority (96%) of N must have been acquired from wet deposition, dry deposition of aerosols, or direct uptake of airborne NH_3 gas (Table 2).

Table 1. Nutrient concentrations (mean ± SE) of oak leaves (n=7) and Tillandsia (n=12) tissue.

Nutrient	Quercus virginiana leaves (mg	*Tillandsia* tissue (mg g^{-1})
C	478.31 ± 2.75	442.07 ± 3.01
N	20.06 ± 0.50	6.02 ± 0.29
P	1.69 ± 0.14	1.00 ± 0.13
K	6.71 ± 0.66	4.31 ± 0.50

Table 2. Different sources of N input and their relative contribution to the total N input to Tillandsia. Total tissue N assimilation was calculated based on the relative growth rate (Garth 1964; Angelini & Briggs 2015).

Source	input	% of total
Total tissue N assimilation	2409.3	100%
N_2 fixation	6.6	0.28%
Dust, excreta and other particles	81.8	3.4%
Estimated wet and dry deposition input	-	96.32%

Deposition

We compared rainfall below oak trees under bare branches to *Tillandsia* covered branches and

found no difference in volume of rainfall over 3 events ($F_{1,12}$=1.363; ns). Cumulative loads of N in this total deposition were around 30 mg N m^{-2}, with a ratio of NO_3:NH_4 of 1:1.5 (±0.4), and loads did not differ between bare branches and *Tillandsia* covered branches ($F_{1,12}$=0.031; ns) (Fig 2). Rainwater N concentrations under trees and in the open field were found to be similar (results not shown). Combining these results with the average total N deposition in rain on the coast of Georgia (1-1.5 kg N ha^{-1} y^{-1} as NH_4 and 1-1.5 kg N ha^{-1} y^{-1} as NO_3; National Atmospheric Deposition Program), we estimate total N load in throughfall deposition to be equal to the N levels outside trees: 1 kg of N-NO_3 and 1.5 kg N-NH_4 ha-1 y^{-1}, adding up to a total 0.25 g N m^{-2} y^{-1} both with high and low *Tillandsia* cover.

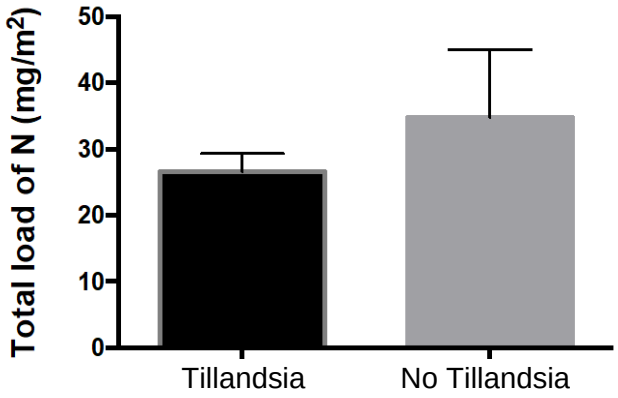

Figure 2. Cumulative N input by deposition after 3 rain events (N=7) ± SE. No significant difference was found between values under Tillandsia clumps compared to those under bare branches.

Isotopic N signature

While leaves of *Q. virginiana* trees had a high isotopic N signature of on average 1.17, *Tillandsia* plant material had a much more depleted value of -10.17 (Table 3), indicating that *Tillandsia* has a different source of N than input of dust or leachates from the tree. N isotope signatures in precipitation in clean air have more similar, depleted values (Moore 1977; Koopmans *et al.* 1997; Liu *et al.* 2013) (Table 3).

Table 3. Isotopic N signature of Tillandsia, Q. virginiana bark and leaf tissue, dust, and rain.

Source	δ 15 N	%N
Tillandsia tissue	-10.17	0.60%
Leaves tree	1.17	1.75-2%
Bark tree (N=3)	1.13	1.18%
Dust	-1.65	1.54%
Rain NH_3 (Moore 1977)	-10.0 (± 2.6)	
Rain NH_4 (Moore 1977)	-1.4 (± 3.5)	
Rain NO_3 (Moore 1977)	-6.6 (± 3.9)	

N availability in soil

Decomposition of *Tillandsia* festoons on the ground resulted in additional leaching of N to the humus layer underneath. In this layer (where the majority of the fine tree roots are found), the plant-available NH_4 concentration was 2 times higher in festoon-covered versus control areas ($F_{1,11}$=6.133; p<0.05) (Fig. 3), while NO_3 concentrations did not differ ($F_{1,11}$=0.000; n.s.; results not shown).

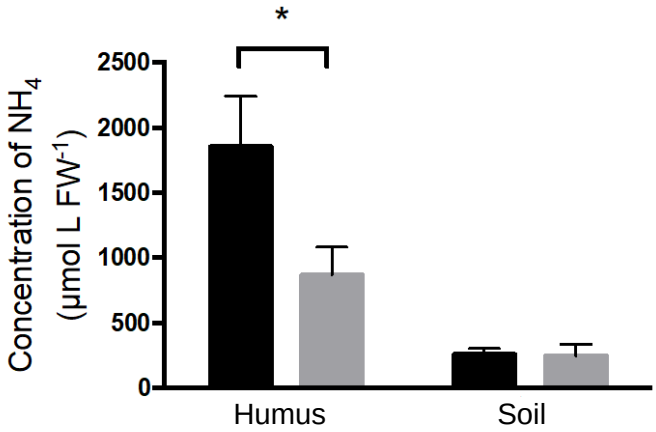

*Figure 3. Plant-available NH4 in the humus layer (N=6) and the soil layer below (N=7) under dislodged, decomposing Tillandsia festoons (black bars) and at spots without decomposing Tillandsia (grey bars). * indicates significant differences at p<0.05.*

N budget tree

To estimate the *Tillandsia* N input to the tree, we calculated the average supply of biomass of decomposing *Tillandsia* under the tree canopy to be 10-60 gDW m^{-2} y^{-1}, depending on the density of *Tillandsia* in the canopy, based on the study by (Angelini & Briggs 2015) whose research was done the same area. Combining this with the N concentration of *Tillandsia* we calculated the contribution of decomposing *Tillandsia* to the N input to the tree (Table 4). The relative N contribution of each source could then be determined by accounting for additional external N sources: N leaching from decomposing leaves from the host tree and throughfall N in deposition. Evergreen trees resorb N during senescence for reuse. Resorption is estimated at 47% of the N in leaves of evergreen trees before shedding (Aerts 1996; Killingbeck 1996). With an N concentration of 20 mg g^{-1} and a deposition of oak leaves of around 80 g DW m^{-2} y^{-1} (Angelini & Briggs 2015) we could calculate the N contribution of senescing oak leaves to the tree (Table 4). Compared to the total N input from deposition and shedding of leaves, *Tillandsia* festoons add 5-24% of N to the total aboveground N input. This can be expected to be even more given that N from decomposing *Tillandsia* is a better N source for tree roots than inorganic N in deposition (Nair *et al.* 2017).

Table 4. External N inputs to solitary southern live oaks.

N input to tree	N (in g m^{-2} y^{-1})	N (in g m^{-2} y^{-1})	% of total
Tillandsia (0.6% N)	0.06 (5%)	0.36 (24%)	5-24
Oak leaves (2% N)	0.75 (64%)	0.75 (50%)	50-64
Throughfall deposition	0.25 (31%)	0.25 (25%)	25-31
Total	1.06 (100%)	1.36 (100%)	

Discussion

Although epiphytes are generally regarded as commensals or parasites with respect to their host trees, here we show that this relationship may also be mutualistic, as the host can benefit from additional slow-release N-inputs, representing 5-25% of the aboveground N input. We also show that *Tillandsia* is most likely directly absorbing this N from atmospheric deposition (dry and wet) or as NH_3 gas, while tree leaching, N_2 fixation and dust collection play little to no role in *Tillandsia*'s N uptake.

N input to Tillandsia

Surprisingly, and in contrast to our hypothesis, N deposition (wet, dry) and/or direct NH_3 uptake seems to be the most important source of N for *Tillandsia*. To verify this, we looked into stable isotope signatures of N inputs to *Tillandsia* to assess the source of N in *Tillandsia*. The external N inputs we measured: tree leaves and bark, dust, and N_2 fixation ($\delta^{15}N = 0$), have much more enriched $\delta^{15}N$ signatures than *Tillandsia* tissue with an isotopic signature of around -10 (Table 3). Therefore, the isotopic signature of these components cannot explain the depletion in *Tillandsia* δ15N. In other words, *Tillandsia* is not significantly absorbing N that may be leaching from the tree itself and it does not seem to negatively affect the N-budget of the tree. Similarly, dust and N_2 fixation seem to contribute only little to the N input.

Other terrestrial plants (Peterson & Fry 1987)as well as NH_4 and NO_3 in rain often have more enriched signatures than the -10 value of *Tillandsia* tissue, depending on their source (Moore 1977; Dillon & Chanton 2005). However, (Hietz & Wanek 2003) found comparably low $\delta^{15}N$ signatures of around -12.4 for atmospheric bromeliad species, like *Tillandsia usneoides*, that seemed to contrast the isotopically enriched rainwater measured in the study with a $\delta^{15}N$ signal of 3.55. On the contrary, N gasses like NH_3 and N_2O were found to have comparably depleted $\delta^{15}N$ signatures as *Tillandsia* (Moore 1977; Felix *et al.* 2014) and it was hypothesized that plants, especially N limited epiphytes, can take up NH_3 gas from the atmosphere (Tozer *et al.* 2005). The isotopic $\delta^{15}N$ signature of atmospheric NH_3 measured close to Sapelo Island (<200 miles distance) is -4.7 and the signal is even more depleted in other measurement sites in the USA (-15.1 on average) (Felix *et al.* 2017). Volatilization of NH_4 in deposition to NH_3 and

diffusive uptake of NH_3 by *Tillandsia* result in further fractionation, leading to more depleted $\delta^{15}N$ signatures (Tozer *et al.* 2005). This may well explain the low $\delta^{15}N$ signatures we found and thus suggests that direct uptake of NH_3 or of volatilized NH_3, and uptake of NH_4 from wet and dry deposition probably represent the most important N input to *Tillandsia*.

N limitation of the host tree

Strikingly, our results show that decomposing *Tillandsia* results in an ecologically relevant additional N input to, and availability in, the forest soil, especially given the fact that the organic humus soil layer contains a large part of the fine oak roots that are taking up nutrients (Mori *et al.* 2009). N has been suggested to be the nutrient limiting biomass production in these systems (Tilman 1986), which was affirmed for red oak by fertilization with N leading to increased basal area (Finzi 2009). The N concentration measured in *Q. virginiana* leaves of 20 mg g^{-1} is indeed in the low (limiting) range (<23 mg g^{-1}) of leaf N concentrations of 4 different European oak species (van den Burg & Schaap 1995; Finzi 2009). Although phosphorus (P) could also be limiting oak growth, N: P ratio of the tree leaves was 12 g g^{-1}, which is generally considered to suggest N limitation (N: P < 14; (Tessier & Raynal 2003; Güsewell 2004; Reich & Oleksyn 2004) rather than P limitation (N: P >20; (Güsewell 2004)). In addition, P concentration in leaves (1.7 mg g^{-1}) is in the saturated/high range of P concentrations in European oaks (van den Burg & Schaap 1995), suggesting that P is not limiting production. So, the increased input of N from decomposing *Tillandsia* festoons to the soil can be expected to be beneficial for growth of its host oak tree, especially since decomposing organic material provides a more easily accessible N source than N in inorganic forms (Nair *et al.* 2017).

Main conclusion: mutualism

Tillandsia receives structure and higher humidity from the oak trees, obviously benefitting the epiphyte (Angelini & Briggs 2015). We here show that the epiphyte obtains its N mainly from deposition, without diminishing the N concentration in rain reaching the soil under the tree, by using NH_3 (direct and volatilized from NH_4) and probably also NH_4 from additional deposition. *Tillandsia* transfers this additional N as a slow-release organic N source, stimulating growth of its N-limited host tree. We show that after dislodging, *Tillandsia*, provides a relevant input of N of up to 24% of the total aboveground N input. This relative N input is much higher than the contribution of epiphytes in humid tropical forest of 8% of N (Tanner 1980; Nadkarni & Matelson 1992). In this way, *Tillandsia* represents an ecologically important N source with a total load of 0.6-3.6 kg N ha^{-1} y^{-1} (Table 4). In trees with high *Tillandsia* cover, the absolute load produced is 3.6 kg N ha^{-1} y^{-1}. In this way, the tree benefits from the additional input of nutrients that the epiphyte can access, demonstrating that *Tillandsia* is a mutualistic symbiont of its host tree.

5. How poaching impacts the regulation of plant abundances in species with complex life cycles

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Vervangen door opgemaakte versie

Abstract

Overhunting is a pervasive threat for mammal communities around the globe. The loss of these mammal populations also mean the loss of their interactions, both mutualistic and antagonistic, with plant communities. An important question is what consequences the loss of mutualistic or antagonistic interactions will have for the success of individual plant species. Many plant species have complex life-cycles with multiple life-stages, where they depend on vertebrate frugivores for dispersal of seeds but are simultaneously limited by seed predators, herbivores and other natural enemies at other life stages. It is both unknown, and challenging to predict, how the deterioration of these relations will interact across the life-cycle of plants and influence plant abundance. Here we propose a simple theoretical framework which predicts how multiple life-stages and vital rates determine plant abundance through their influence on two key statistics: low-density population growth rates (the 'invasion growth rate') and strength of density-dependent population growth ('stabilization'). The model predicts that hunting will increase plant abundance through lack of dispersal, unless this is compensated by increased density-dependent regulation. We then continue to test our framework in a case study on a well-studied, mammal-dispersed palm species, Attalea butyracea. In the case study, we quantify the full life-cycle of this palm along a gradient of population abundances in a hunted and a protected forest. We show that loss of dispersal services increases low-density population growth rates within local populations, and that negative density-dependent enemy responses do not compensate for the increased seed densities effectuated by failed dispersal. In the end, increased invasion growth rates, and net decreases in density-dependent regulation, result in predicted five-fold increases in plant abundances. We discuss our result in relation to previous work and conclude that negative density-dependent interactions seldom compensate for the increased local seed densities caused by the loss of vertebrates. Using our framework, we predict that, contrary to expectations, the local dominance of animal dispersed species increases as a result of hunting, which may still have far-reaching effects on vegetation heterogeneity and biodiversity but the opposite direction to what is commonly assumed.

Introduction

Overhunting is a pervasive threat to many large vertebrate species, especially in the tropics (Corlett 2007; 2007; Harrison *et al.* 2013) where hunting pressure is often unsustainably high (Wilkie *et al.* 2011). This can lead to so-called 'empty forests' in which the vegetation appears intact but larger vertebrates are absent (Redford 1992; Harrison 2011). A great concern beyond the loss of the vertebrates themselves is the loss of their interactions with the vegetation (Janzen 1974). These losses may have important consequences for patterns of recruitment, the competitive balance between plant species, and ultimately the species composition, structure and functioning of forests (Stoner *et al.* 2007; Harrison *et al.* 2013; Dirzo *et al.* 2014; Osuri *et al.* 2016).

Overhunting is thought to particularly affect the recruitment of plant species that depend on large vertebrates for seed dispersal (Caughlin *et al.* 2015). Poor seed dispersal in sites with heavy hunting has been documented for a variety of species (reviewed in Wright *et al.* 2007a). Seed dispersal is critical for escaping natural enemies concentrated around parents (Comita *et al.* 2014) and for reducing kin competition (Howe & Smallwood 1982; Nathan & Muller-Landau 2000). Hence the general prediction is that overhunting increases the impact of negative density-dependence and reduces recruitment success (Brodie *et al.* 2009; Wotton & Kelly 2011; Caughlin *et al.* 2015). This would ultimately cause populations of animal-dispersed plant species to decline, in favor of plant species that do not depend on larger vertebrates for seed dispersal, such as wind-dispersed species (Muller-Landau 2007; Wright *et al.* 2007a; Effiom *et al.* 2013).

Hunting, however, also affects the abundance of seed predators or herbivores such as large rodents and ungulates, which may consume important numbers of seeds or seedlings. The net consequences of hunting for the recruitment success of animal-dispersed plant species will thus depend on the relative impact of hunting on mutualists as seed dispersers, antagonists such as seed predators as well as the relative importance of the interaction between mutualist and antagonists. Defaunation may thereforenot always have a negative impact on mammal-dispersed species, and examples exist of field studies in which hunting apparently favors mammal-dispersed species (Dirzo *et al.* 2007; Wright *et al.* 2007a). Nevertheless, the underlying synergistic effects of seed-disperser and seed-predator loss have rarely been investigated (but see Culot *et al.* 2017). In conclusion, the net impacts of hunting depend not only on how it affects seed dispersal, but also on whether it aggravates or alleviates interactions with natural enemies.

Many mammal-dispersed plant species also have complex size-structured life cycles, and positive or negative effects at one life stage could be offset by an opposite effect at a later stage

(see e.g. Visser *et al.* 2016). Such interactions across the life cycle are challenging to predict as different life stages can make extremely unequal contributions to plant fitness (see e.g. de Kroon *et al.* 2000). What is lacking is 1) a theoretical framework of how dispersers and natural enemies interact to determine the local abundance of plant with multiple life-stages and 2) an empirical evaluation of such a theoretical framework on a population level (i.e. full life-cycle). Both 1 and 2 are needed before we can start to understand under which conditions defaunation leads to the local success or failure of plants species with multiple life stages and complex life histories.

Here we introduce such a theoretical framework to both better understand and measure the impact of hunting on density-dependent population growth for species with multiple life stages. We then set out to empirically evaluate our theoretical framework. A broad empirical evaluation is however challenging as previous work rarely quantifies dispersal, only recorded density dependence at small spatial scales with incomplete life-cycles, or measured density-dependence and vital rates in intact forests and/or simulated the effects of hunting (Brodie *et al.* 2009; Terborgh 2013; Caughlin *et al.* 2015). Therefore, no study ever actually measured how full-life cycle dynamics change across a gradient of population densities in both defaunated and intact situations. This is why we evaluate our framework with a case study where we measure density-dependent interactions across the entire life-cycle, for all vital rates, in a common tree species along a gradient of population density, and at spatial scales relevant to the population (see e.g. Schupp 1992; Visser *et al.* 2011) in both a protected and hunted site in the Republic of Panama. Finally, we discuss the generality of our case study and framework with references to the literature. We start by describing our theoretical framework and using it to generate testable hypotheses which we test with our case study.

Theory

We explore how plant species abundance and population dynamics are jointly influenced by seed dispersal, density dependence and density-independent factors, using a simple stage-structured population model (Fig. 1A). The model describes population change in single local (i.e. spatially distinct) population using the following equations:

$$\frac{dN_j}{dt} = (1-e)rN_a\left(1-\frac{(1-e)rN_a}{K}\right) - N_j$$

$$\frac{dN_a}{dt} = mN_j - (1-s)N_a$$

Equation 1

Here, N_j and N_a are the number of juveniles and adults, respectively. Recruitment depends on per capita seed production (r), the rate at which seeds leave the population through dispersal

which lowers seed densities in the (local) population through emigration of seeds (e), and the strength of negative density-dependent factors that influence the survival of seeds and the recruitment of seedlings (K). In the model the net attack by natural enemies is a function of the density of seeds which follows empirical evidence (Bagchi *et al.* 2010; Comita *et al.* 2010; Bagchi *et al.* 2011). The dynamics of the adult stage is affected by adult survival (s) and the maturation rate of juveniles (m), which either mature or die. In the model, vital rates of later life stages are assumed to be density independent - as is common in trees (e.g. Zhu *et al.* 2015; Zhu *et al.* 2018). For simplicity, we ignore immigration from outside the population and do not allow juveniles to survive though we note this can be added without changing the model's basic behavior.

In this theoretical framework, the expected local plant abundance (or equilibrium density; $\widetilde{N} = \widetilde{N_J} + \widetilde{N_a}$) is determined by two key metrics: 1) the invasion growth rate ($\lambda_{N=0}$) or the per-capita population growth rates at low density and 2) the rate of stabilization ($\frac{d\lambda}{dN}$), or the decrease in the per-capita population growth with increasing density (Fig 1B). Density-independent factors (e, r, m & s) influence both the height of $\lambda_{N=0}$ and the strength of $\frac{d\lambda}{dN}$, while density-dependent factors (K) only affect $\frac{d\lambda}{dN}$ (see Fig. S4). The theoretical impact of hunting on local plant abundances ($\widetilde{N}$) will therefore depend on how hunting influences both the invasion growth rates $\lambda_{N=0}$ and stabilization $\frac{d\lambda}{dN}$. In reality, hunting may affect multiple life-stages and vital rates (e.g. Wright *et al.* 2007b; Brodie *et al.* 2009) and therefore we must determine how hunting affects all density-dependent and independent vital rates across all life-stages before we can understand it's impact on the invasion growth rate and strength of stabilization, and ultimately predict the consequences of hunting for plant abundance.

The model allows us to explore how hunting may impact plant abundance when it acts on a single or multiple vital rates and generate basic scenarios : 1) hunting diminished disperser densities, directly lowering *e* which, all else being equal, increases $\lambda_{N=0}$ and results in higher expected local plant abundances (Hunting scenario 1, i.e. H1 in Fig. 1C). However, decreased dispersal also results in greater aggregation of seeds (Caughlin *et al.* 2015) to which natural enemies (i.e predators or pathogens) may show both numeric and functional responses (sensu Holling 1959, 1961). When enemies are density responsive, this, synergistically may result in greater attack rates and hence lower K values at poached sites. This by itself, leads to a stronger rate of stabilization (i.e. more negative) and lower plant abundance at least when K is lowered substantially (see Scenario H2, Fig. 1C). Finally, hunting may not only affect disperser densities but also diminish seed predator densities, or even release of top-down control of pests (e.g. Terborgh & Estes 2010; Visser *et al.* 2011). Hence, hunting could result in a both lower e and

higher K leading to increased $\lambda_{N=0}$ and weakened $\frac{d\lambda}{dN}$ and resulting in greater local plant abundances ($\tilde{N}$) (see scenario H3, Fig. 1C).

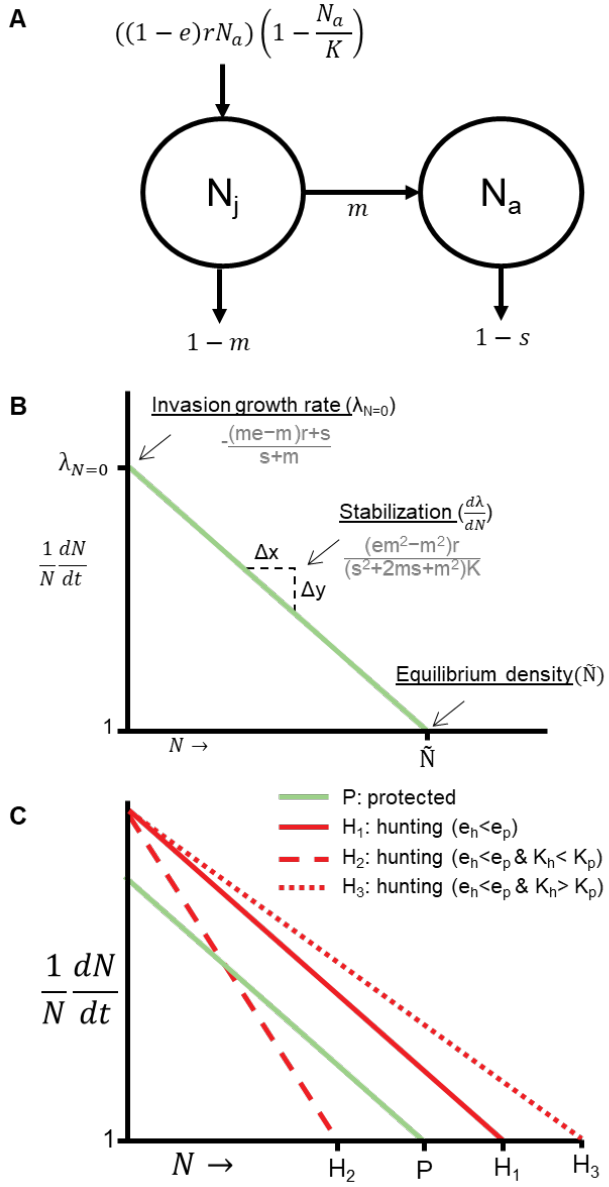


Figure 1. The theoretical impacts of hunting on plant abundance depends on how hunting affects plant species invasion growth rates ($\boldsymbol{\lambda_{N=0}}$) and population level negative density dependence (termed stabilization $\frac{d\lambda}{dN}$;). A) Simple plant population model where local juvenile densities (N_j) depend on the total amount of seeds produced locally (rN_a), local loss of seeds through emigration (e) by dispersal and total (density-dependent) effects of natural enemies (i.e. host specific enemies; K). Local adult densities (N_a) depend on the maturation of juveniles (m) and adult survival (s). B) The predicted relationship between local population-level growth rate and population densities ($\tilde{N}$), where the final abundance (equilibrium density N when $\lambda = 1$) depends on the height of invasion growth rate ($\lambda_{N=0}$) and the rate of stabilization ($\frac{d\lambda}{dN}$). C) Theoretical impact of hunting depend on whether hunting only impacts dispersal (lowering e; scenario H1 and solid red line), or also increases natural enemies attack (lowering K; scenario H and dashed red line), or hunting impacts both dispersers and seed predators (decreasing e and increasing K; scenario H3 and dotted red line).

Case study

To test the theoretical predictions derived above, we focus on a model system of the large-seeded Neotropical palm *Attalea butyracea* (henceforth *Attalea*). *Attalea* is well studied in all aspects of its life-cycle from seed dispersal (Jansen *et al.* 2014), seed predation (Visser *et al.* 2011) to density-dependent population dynamics (Visser 2016). *Attalea* is an arborescent palm which relies on frugivorous and granivorous mammals for seed dispersal (Jansen *et al.* 2014) and suffers host-specific seed predation from parasitoid *Bruchinae* beetles (hereafter bruchid beetle) and generalistic seed predation by large rodents (Visser *et al.* 2011). The host-specific bruchid beetle have been shown to suffer top-down control though predation by large rodents (Visser *et al.* 2011).

Based on expected trends from the literature and our theoretical model predictions we hypothesize that hunting reduces seed dispersal (Peres & Palacios 2007; Markl *et al.* 2012; Caughlin *et al.* 2015; Kurten *et al.* 2015) and rodent predation (Wright *et al.* 2007a; Kurten *et al.* 2015), increases local seed densities as well as the strength of negative effects of density (due to host-specific parasitoid beetles being less controlled by rodents; Visser et al 2011), which synergistically lead to greater per-capita mortality of seeds and seedlings (Janzen 1970), and eventually reduces the long-term expected abundance of *Attalea* (corresponding to H2 in figure 1C). Scenario H2, under our theoretical framework, is also the scenario most commonly assumed in the literature (Freckleton & Lewis 2006; Bagchi *et al.* 2010; Bello *et al.* 2015; Osuri *et al.* 2016; Peres *et al.* 2016). We specifically test the following hypotheses:

(1) Hunting reduces seed dispersal distance and emigration rates (<e) leading to local accumulation of seeds and higher local seed densities.
(2) Hunting strongly aggravates host-specific seed predation and population-level negative density dependence of seedling recruitment and survival (<K);
(3) Loss of seed dispersal due to hunting (smaller e) results in: 3a) greater invasion growth rate ($\lambda_{N=0}$) at hunted sites, but this effect is over-compensated by far greater rates of seed predation and lower seedling recruitment and survival (lower K) leading to 3b) stronger stabilizations rates ($\frac{d\lambda}{dN}$) and eventually 3c)lowered local abundances (Ñ) as is commonly expected.

To test these predictions, we compared the fate of individuals at different life stages – from seed to adult – along a gradient of population density in a protected and in a hunted portion of the same forest complex. We first assessed how hunting affected population-level density dependence of dispersal and seed predation by rodents and bruchid beetles. We then quantified density-dependent survival, growth and reproduction across the entire full-life cycle of *Attalea*. Matrix population models (Caswell 2001) were then used to integrate all previously quantified rates and estimate how these together determined invasion growth rates, density

dependence of population growth (stabilization) and ultimately changes in expected abundances. To our knowledge, our case study is the most comprehensive study done to date on the effects of defaunation on population dynamics for any plant species.

Methods

Case study overview

Our goal is to document how the loss of seed dispersers/predators affects the population dynamics of *Attalea butyracea* through interaction with density-dependent mechanisms. We do this in six steps.

1) Quantify how loss of dispersers affects density-dependence of seed dispersal distances by fitting dispersal kernels to field data using inverse modelling.
2) Quantify how mammal (rodent) seed predators affect density-dependence of seed mortality and whether seed predation by specialized insect natural enemies compensates for the loss of rodent seed predators.
3) Quantify how altered rates of seed dispersal and predation affect local seed and seedling densities, through density-dependence in seed to seedling transition rates and subsequent seedling survival.
4) Quantify all remaining vital rates (growth, survival and reproduction) across the life-cycle of *Attalea* using vital rates models that include adult density and site effects.
5) Integrate all previously fit vital rates models in a density dependent population model, to calculate site effects on the invasion growth rates ($\lambda_{N=0}$), equilibrium density (Ñ) and the change in λ at the equilibrium density (a measure of stabilization; $\frac{d\lambda}{dN}$).
6) Estimate the relative importance of all site effects quantified above to differences in population dynamics, and quantify which are due to hunting.

Throughout the manuscript we use capital Roman letters to denote functions, bold letters for matrices, Greek letters for parameters and lowercase Roman letters for variables (life stage, size, time, density etc.). All models were run in R 3.4.3 (R Development Core Team 2016).

Study system

Fieldwork was conducted in 17 4-ha plots in the Republic of Panama at one protected site, Barro Colorado Island (BCI), and one hunted site, Pipeline road (PLR). A total of 10 plots were established on Barro Colorado Island (BCI; 9°9'N, 79°51'W), a 1560-ha island in the Gatun Lake section of the Panama canal. Logging and hunting is prohibited on BCI, and game wardens strictly enforce these rules. Annual rainfall averages 2650 mm (1929-2016) on BCI.

A total of 7 plots were established on PLR, which lies adjacent to the Panama Canal at approximately 15 km from BCI. Logging and hunting are prohibited on PLR, though illegal hunting is common due to easy accessibility and lower enforcement, resulting in considerably lower mammal densities (Wright *et al.* 2000).

Plots on both BCI and PLR were established in secondary tropical forests, have a dry season spanning December to April, and are situated on volcanic material (Stewart *et al.* 1980), weathered into clay rich, yellow-brown Alfisols (Yavitt 2000). Tree species composition and biodiversity are similar at both sites (Wright *et al.* 2000).

Attalea butyracea (Mutis ex L.f.) Wess. Boer is a monoecious palm that reaches heights of ~30 m (Desteven *et al.* 1987) and is the 17th most abundant species among the 227 'hyper-abundant species' that together comprise >50% of all stems in Amazonia (ter Steege *et al.* 2013). The life-cycle of *Attalea* palms is illustrated schematically in Figure S1. Reproductive palms produce up to 3 infructescences each year, with between 100 to 600 fruits per infructescence (Desteven *et al.* 1987; this study). Fruits are heavy (>10 g; Foster *et al.* 1982) and large (3-5 cm; Wright 1983), and have a soft fruity mesocarp around a hard endocarp which is durable that decomposition takes several years (Wright 1983).

Numerous mammal species are known to consume and disperse *Attalea* fruits, including tapirs, coatis and primate species (see Jansen *et al.* 2014 for an overview), however only three local vertebrate seed predators are capable of opening the hard endocarps. These include Red-tailed squirrels (*Sciurus granatensis*), the Central American agouti (*Dasyprocta punctata*), and the Central American spiny rat (*Proechimys semispinosus*). The characteristic scars of spiny rats were not encountered in this study and are therefore no longer considered. In addition, two bruchid beetle species, *Speciomerus gigantus* [Chevrolat] and *Pachymerus cardo* [Fahraeus] prey on *Attalea* seeds. These beetles have no other local host species, laying eggs solely on *Attalea* fruit, after which larvae burrow through the endocarps and consume the endosperms (Wright 1983).

Field measurements

We located areas with contrasting population densities at both sites, and established square 200×200 m (4-ha) plots that ranged twenty-fold in adult *Attalea* density on BCI (1.25–24.4 palms ha^{-1}), and four-fold on PLR (4-16 palms ha^{-1}). Each plot had one reproductive *Attalea* at its center and was separated by at least 300 m from another plot. Plots were divided into a central one-hectare, in which adults, juveniles and seeds were monitored, and a three hectare buffer in which only adults were censused. The buffer was included to minimize the influence of immigrant seeds from unknown adults (more details in Visser *et al.* 2011; Jansen *et al.* 2014; Fig. S2).

Adult census

Between October 2007 and February 2008, every *Attalea* palm with a bole height >1.3m was tagged and mapped. For each individual we recorded the Dawkins's crown position index (*c*), which estimates the crown position relative to those of neighboring trees with a 5 point score (Dawkins & Field 1978); (1) No direct light, crown plan entirely shaded both vertically and laterally; (2) Lateral light, crown plan entirely vertically shaded but exposed to some direct light laterally; (3) Partial overhead light, crown plan partly exposed vertically but partly shaded vertically; (4) Full overhead light, crown plan fully exposed vertically but adjacent to other crowns within 90◦ inverted cone subtended by the crown base; (5) Emergent crown, crown plan fully exposed vertically, and free from lateral competition with the 90◦ cone. CP estimates are repeatable and display strong correlation with canopy openness or incident radiation (see Clark & Clark 1992; Clark *et al.* 1993; Davies *et al.* 1998). Reproductive individuals were identified by the presence of infructescenses and inflorescences (which remain on the palm for a year). In June 2012 we conducted a full re-census of all previously mapped individuals, as well as mapping any new recruits (palms > 1.3 meters in height).

Seed census

We investigated seed fate for endocarps collected from the forest floor and topsoil between January and August 2008. We placed 32 1-m^2 seed-quadrats in computer-generated random locations in a stratified random manner in each plot, with two quadrats in each of the sixteen 25×25 m subplots in the central hectare of each 4-ha plot (544 seed-quadrats total). If a rock, tree or debris covered a randomly selected point, the quadrat was placed as close as possible to the randomly selected point in a randomly generated direction (details in Visser *et al.* 2011). The surface and top 5 cm of soil of each quadrat were thoroughly searched for endocarps using a small rake. Seed predation and survival can be inferred from predation scars on the endocarps (Visser *et al.* 2011). Predator-specific seed fate can accurately be distinguished by size, location and shape of scars (see Silvius & Fragoso 2002; Visser *et al.* 2011 for details). Seeds were scored for rodent bite marks, bruchid larvae entrance and exit holes or both to measure total predation percentages. We excluded fresh endocarps from the early fruiting season of 2008, as well as endocarps so old they could be crushed by hand as these could obscure rodent and bruchid scarring patterns. Endocarp densities reflect the sum of the previous three fruiting seasons (see Visser *et al.* 2011; Jansen *et al.* 2014).

Seedling census

Between January and April 2008, we mapped all palms < 1.3 meters in height within subplots in each plot. Seedling densities differed greatly between sites, and further subsampling was necessary on PLR. The sampling protocol included the following steps. First, we divided the

central part of each 4 ha plot (the central 100×100 m), into 16 subplots of 25×25 meters. We then randomly selected one of the four inner subplots and three of the 12 outer subplots. All *Attalea* individuals within these plots were tagged with vinyl loop tags, and we recorded the length of the longest leaf, as well as the number of simple or complex leaves. When seedling densities were roughly 100-250 seedlings per subplot, the subplot was divided into 4 sub-quadrats of 12.5x12.5m of within which 2 sub-quadrats in a diagonal line were used to map and record all *Attalea* individuals. When the seedling density was estimated to exceed 250 individuals per subplot, the subplot was divided into 16 sub-quadrats of 6.25x6.25 meters of which 4 were censused in a diagonal line across the subplot (see Fig. S2 for a graphical representation of plot lay-out). In June 2010 and June 2012, all seedling plots were revisited, surviving individuals were measured again and new recruits were mapped.

Quantifying dispersal

We used inverse modelling (IM) to estimate dispersal distance from the post-dispersal distribution of endocarps in each of the 4-ha plots. IM methods have been described in detail elsewhere (Ribbens *et al.* 1994; Muller-Landau *et al.* 2008; van Putten *et al.* 2012). In short, IM models the spatial distribution of seeds in an area as the superposition of the seed-shadows of all adults within the area. Seed shadows are modelled as the product of each trees estimated seed production and a dispersal kernel, the two-dimensional probability distribution of seed densities around its source. The expected number of endocarps at each 1-m^2 quadrat is the sum of expected contributions of all adults on the 4-ha plot, and from adults outside the plot under the assumption that endocarp production per unit area off-plot equalled that on the plot (Muller-Landau *et al.* 2008). Observed endocarp numbers were assumed to follow a negative binomial distribution around their expected (Clark *et al.* 1999).

To test for the influence of adult density and hunting on seed dispersal distances, we used the best fitting seed shadow model from Jansen et al. (2014) for the same study system, which uses a 1-parameter 2Dt function with a shape parameter fixed at 2 and free scale and seed production parameters. We fit this model for each plot separately. The parameter space was searched using generalized simulated annealing, which is a robust method for finding global maxima (Xiao *et al.* 2013). We estimated standard errors for each model parameter through numerical approximation of the second partial derivative matrix of the log-likelihood function at the maximum-likelihood estimate (the Hessian). The inverse of the Hessian is an estimate of the variance–covariance matrix (Bolker 2008). We used the fitted seed shadow to calculate seed production and median dispersal distances for each plot.

Effects of hunting and density-dependence across the life cycle

The Attalea life cycle

We structured the *Attalea* life cycle in three distinct stages following the classification by Visser (2016; Fig. S1): 1) seedlings with simple leaves (stage s), 2) basal rosettes (stage r) with compound leaves, and 3) individuals with woody trunks with a distinct crown position (c)(Clark & Clark 1992) . This morphological classification coincides with significant shifts in either growth, survival or reproduction.

Density-dependent vital rate models

We fitted density-dependent vital-rate models, including growth, survival, and reproduction at each of the three life stages. Models were functions of adult density (a in ha^{-1}) and site (z, hunted or protected site) for seedlings and rosettes, and functions of crown position index (c), adult density and site for stemmed individuals. We evaluated multiple models for each vital rate, and evaluated both the best model and averaged models. The procedure to fit each vital rate functions is described briefly below. Unless specified, all vital rate models were linear, where predictions are functions of adult density (a) and site (z). Models included random intercepts for census period (γ_{year}) when appropriate (Table S1). Models were selected based on AIC scores, and to prevent exclusion of models with similar fits (e.g. $\Delta AIC < 10$) we performed model averaging on model parameters (Anderson & Burnham 2002; Whittingham *et al.* 2006; Bolker *et al.* 2009). Model averaging is expected to provide a more robust basis for inference and prediction in cases where multiple variables influence the response variable (Grueber *et al.* 2011). We averaged parameters over all models having AIC weights > 0, and assigned the value zero wherever parameters were absent from models – which is a conservative approach to model averaging (i.e. leading to lower effect sizes) (Anderson & Burnham 2002; Grueber *et al.* 2011). Confidence intervals for each weighted parameter were estimated following Buckland et al. (1997). As a robustness check we also compared results obtained from model averaging to results obtained by simply selecting the best model (i.e. the model with the lowest AIC).

Annual Seed production $F(a,z)$ was estimated from the inverse modelling described above for each plot, and related to adult density and site with linear regression models. Models are given in Table S1.

Seed predation $P(a,z)$ was estimated from predator-specific scars on *Attalea* endocarps.We related the probability of seed predation by rodents, bruchids and total seed predation to adult density and site using information on scarred seeds and total seeds across the 32 seed-quadrats in each plot. We then fitted logistic models to test how seed fates changed with adult density

for the following seed fates: total seed mortality, total rodent attack and total bruchid attack. Models are given in Table S1.

Seedling establishment probabilities, E(a,z), were calculated by dividing the density of newly recruiting seedlings at each 2-year census by the 2-year density (m^2) of seeds that escaped predation – defined as having no scarring - for each plot. Seedling establishment probabilities were then linearly related to adult density and site effects.

The *probability of reproduction*, R(c, a, z), *survival* S(c, a, z) and *stage transition* models that describe the probabilities of transitioning from the seedlings stage (s) to the rosette stage (r) ($T_{s\rightarrow r}$ (a,z), *and* $T_{r\rightarrow h}(a,z)$) were fit using logistic models as with seed predation (Table S1). Transition probabilities between crown positions for stemmed palms $T_{c1i\rightarrow c2j}$ (c,a,z) were modelled with multinomial regression as a function of initial crown position ($c_{1,i}$), density (a) and site (z).

Integrating hunting and negative density-dependence across the life cycle

After all vital rate functions are fit, the net effects of both hunting and density dependence on a single currency (e.g. population growth) can be calculated by combining all regression models in a matrix projection model. We used a density-dependent matrix model, which predicts the size-structured distribution of individuals at time t+1 $\mathbf{W_{t+1}}$, as a function of the size-structured distribution of individuals at time t $\mathbf{W}_t$ and a matrix of transition probabilities $A_{(a,\,z)}$: $\mathbf{W_{t+1}} = \mathbf{W}_t\mathbf{A}_{(a,z)}$. Where t+1 is a 2-year time step, corresponding to the seedling census, $\mathbf{A}_{a,s}$ is a 7x7 square Markov transition matrix dependent on adult density (a) and site (z) and $\mathbf{W}$ a 1x7 vector of population densities in each stage class. The matrix $\mathbf{A}_{a,z}$ is further defined as:

$$\boldsymbol{A}(a,s) = \begin{bmatrix} H_s(a,z) & 0 & B_{c1\rightarrow s}(a,z) & \dots & B_{c1\rightarrow s}(a,z) \\ G_{s\rightarrow r}(a,z) & H_r(a,z) & 0 & \dots & 0 \\ 0 & G_{r\rightarrow c1}(a,z) & H_{c1}(a,z) & \dots & G_{c5\rightarrow c1}(a,z) \\ 0 & 0 & \vdots & \ddots & \vdots \\ 0 & 0 & G_{c1\rightarrow c5}(a,z) & \dots & H_{c5}(a,z) \end{bmatrix} \quad \text{(eq. 2)}$$

where the functions $H_i(a,z)$ and $G_{i->j}(a,z)$ represent the rates of stasis and growth which together describes all possible transitions between stages i and j at a given adult density (a) and site (z) – while B describe the production of new individuals in stage s by individuals in stage c_i. Functions H, G and B are in turn constructed from the previously described vital rate functions as shown in table S2. Matrix $\boldsymbol{A}$ is then used to numerically simulate density-dependent per capita population growth from which the following statistics can be estimated for both protected and hunted sites:

Invasion growth rates, $\lambda_{N=0}$, are estimated as the rate of λ when $N=0$, which represents the per capita increase when the focal species is rare.

Equilibrium densities, $\tilde{N}$, are the adult palm densities (N_a) when the asymptotic λ was 1.

Stabilization, $\frac{d\lambda}{dN}$, is the estimated average slope of λ over N_a between 0 to Ñ. Which was numerically estimated, and measures of how strongly the population is regulated.

Confidence intervals were calculated for all three statistics, by sampling from the weighted and theoretically normal (Ott & Longnecker 2001) sampling distributions of each model parameter, and recalculated each statistic 1000 times. This incorporates uncertainty in all lower-level parameters.

Relative importance of different vital rates in explaining site differences

To determine which vital rates were most influential in explaining population-level differences between sites, we iteratively replaced vital rates from the hunted site with their protected equivalent. This reveals the magnitude and directional impact of each vital rate specifically towards differences in the three statistics ($\lambda_{N=0}$, $\frac{d\lambda}{dN}$, $\tilde{N}$). This analysis reveals the degree to which site differences can be attributed to hunting-impacted vital rates or other site differences (e.g. edaphic conditions).

Results

4415 seedlings, 3051 rosettes and 2015 adults were censused between 2008 and 2012 (Table S3) in a total of 68 ha of forest. A total of 65 different models were fit which together resulted in 12 vital rate models that quantify *Attalea* full life-cycle in both the protected and hunted sites. Results were qualitatively similar when only using the single best model (Fig. S3). We discuss each model below following the life cycle of *Attalea* from seeds to adult palms.

The fate of seeds

Inverse modelling estimates of number of seeds produced per reproductive palm were not significantly related to adult density or site (Fig. 2A, Table S1). However, the estimated median seed dispersal distance was strongly negative density dependent in both protected and hunted areas (Fig. 2B, Table S1, $T_{2,13}$= -2.41, p=0.03) with dispersal distances being approximately 5.7 times lower overall in hunted areas (Table S1, $T_{2,13}$= -4.95, p< 0.001). The decreasing seed dispersal distances resulted in increasing seed densities with adult densities ($T_{2,13}$= 5.99, p< 0.001) that were a factor 7.2 greater in hunted areas (Fig. 2C, Table S1, $T_{2,13}$= 5.81, p< 0.001). Variation in mean seed densities across sites and plots was explained well by the inverse model,

with estimated median dispersal distances and fecundity explaining 81% of the variation among plots.

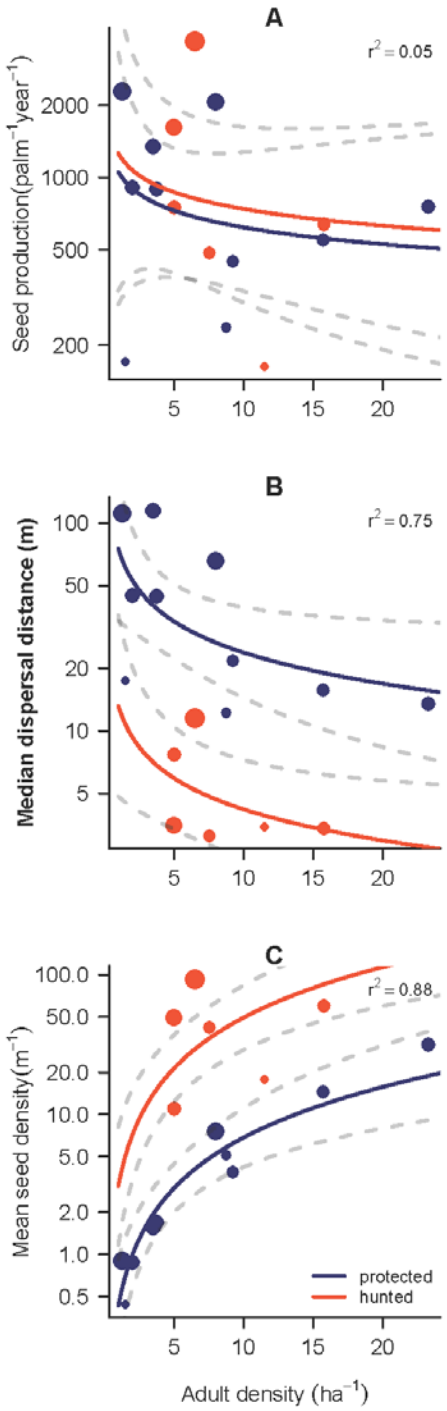


Figure 2. Seed production, median dispersal distance and observed seed densities in hunted (red) and protected areas (blue) as a function of adult density. Seed production A) together with negative density dependent dispersal and failed dispersal in the hunted site B), created positive density dependence of seed densities on adult density, with 620% greater seed densities in poached sites despite almost no differences in seed production between sites. Note the logarithmic scales of the response variables.

Predator-specific scars on endocarps (i.e. rodent or bruchid beetle predation) revealed strong differences between sites in seed predations patterns. Scarring by rodent seed predators were found on 74% of seeds in protected plots but only on 6% of the seeds in hunted plots: seed predation by rodents was therefore 11.7 times lower in hunted areas. Host-specific bruchid beetle attack increased from 26 to 45% with increasing adult densities in protected sites, and roughly doubled in hunted sites, ranging between 79 to 82% (Table S1, $z=-2.82$, $p<0.01$). Note that the proportion of rodent and bruchid attack rates in the protected sites is greater than the total mortality as rodents also consume bruchid larvae (see Visser *et al.* 2011 for details). The increased bruchid predation in hunted areas resulted in the proportion of total predated seeds being slightly higher (1.08 times) in hunted than in protected sites (Fig. 3C and Table S1, $z=2.65$, $p<0.01$).

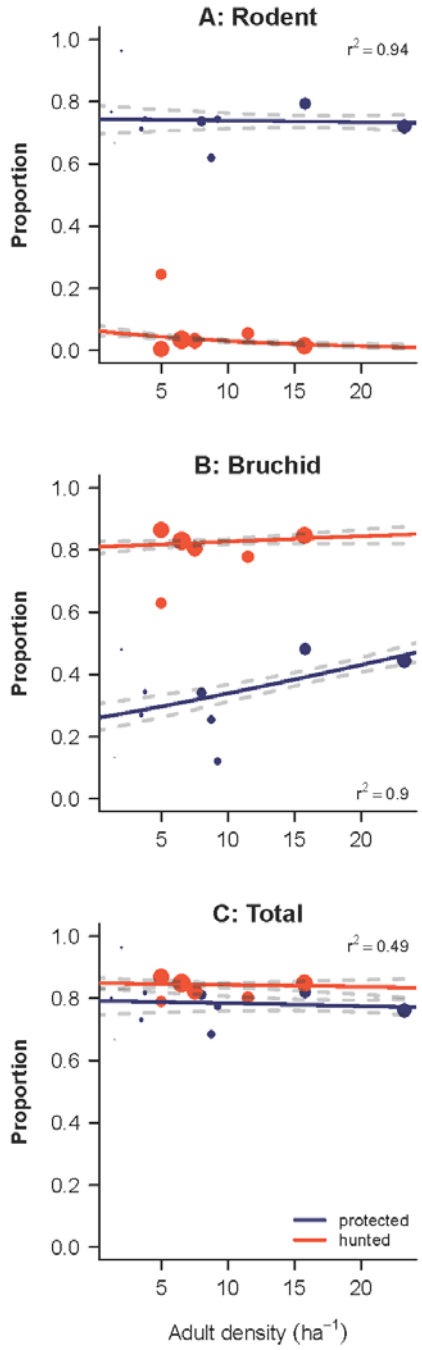


Figure 3. Density dependent seed fate in hunted (red) and protected (blue) areas. Seeds were predated by A) rodents and B) bruchid beetle larvae, which resulted in C) total mortality of seeds by predation.

The fate of recruits and seedlings

The per seed probability of establishing as a seedling (seed-to-seedling transition probabilities) for seeds that escaped predation was strongly negative density-dependent (Fig. 4A, Table S1, z=-3.24, p<0.01) with the slope over adult density not differing among sites (Table S1, z=-0.014, p=ns). Seedling survival was independent of adult density in protected sites with 74% surviving overall. In hunted sites, however, seedling survival was negative density dependent, with survival ranging from 70% to 62% at low to high densities (Fig. 4B, Table S1, z=-5.73,

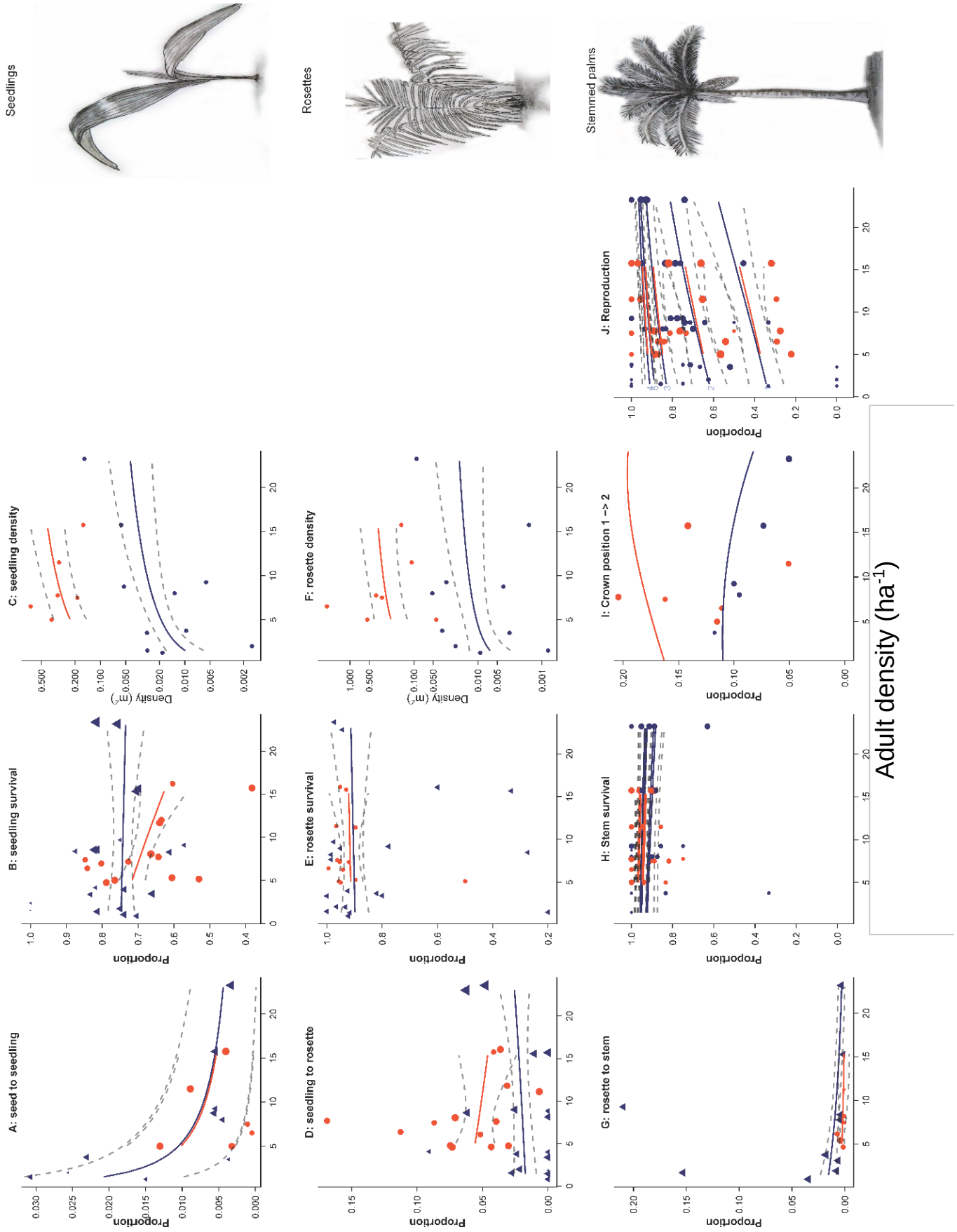
p<0.001). Resulting seedling densities were ~10 times higher in hunted compared to protected areas (Fig. 4C).

The fate of older life-stages

Seedlings were found to transition to the rosette stage at a greater rate in the hunted site and had a slightly higher chance of survival as a rosette, although both differences were not significant (Fig. 4D-E, Table S1). Rosettes transitioned to the stemmed palm stage at very low rates, with on average 1.7% of rosettes transitioning to the stemmed stage every 5 years at protected sites. In the hunted site transition rates were approximately 3 times lower at 0.5% on average (Fig. 4G, z = -0.310, p=ns). Crown position influenced both stem survival rates and reproduction (Fig 4H,J, Table S1) with little differences among sites. Transition probabilities between crown position states were significantly differed among sites (Fig. 4I). To conserve space, Figure 4I only shows the most numerous transition observed, between crown position 1 and 2 (other transition rates are given in Table S4).

On right hand page:

Figure 4. *Density dependence and site differences in the vital rate fits of seedlings, rosettes and stemmed stages of Attalea palms at a protected (blue lines) and hunted site (red lines) in Panama. Panels show A) the 2-year rate of seed-to-seedling transition, B) the 2-year seedling survival rate, C) observed seedling densities, D) the 2-yr rate of seedling-to-rosette transitions, E) the 2-yr rosette survival, F) observed rosette densities, G) 5-yr rate of rosette-to-stem transition, H) 5-yr stem survival rate, I) 5-yr Crown position transition rates for crown position 1-2 the most numerous transition, J) Reproduction probabilities per crown position.*


Seedlings
Rosettes
Stemmed palms
A: seed to seedling
B: seedling survival
C: seedling density
D: seedling to rosette
E: rosette survival
F: rosette density
G: rosette to stem
H: Stem survival
I: Crown position 1 –> 2
J: Reproduction
Proportion
Density (m²)
Adult density (ha-1)

Population-level effects of adult-density and hunting

Density-dependent matrix models for hunted and protected populations including all site- and density-dependent vital rates from the averaged models, showed clear differences between hunted and protected sites in the invasion growth rates and equilibrium densities. Both hunted and protected populations showed strongly negative density-dependent population growth rates (Fig 5A), with the average slope (defined as the strength of stabilization here) trending to be more negative in the protected site, but this difference was not significant. The invasion growth rate ($\lambda_{N=0}$), the annual per-capita increase in population size at low density (N = 0), was estimated to be 1.12 at protected populations (bootstrapped CI: 1.08-1.18), and significantly higher at 1.27 (CI 1.19-1.38) at the hunted populations (Fig 5B). Stabilization ($\frac{d\lambda}{dN}$), the average rate of the change of λ from 0 to Ñ, was -0.062 in protected areas (CI -0.11 - 0.031), while -0.032 in the hunted site (-0.047 - -0.021).

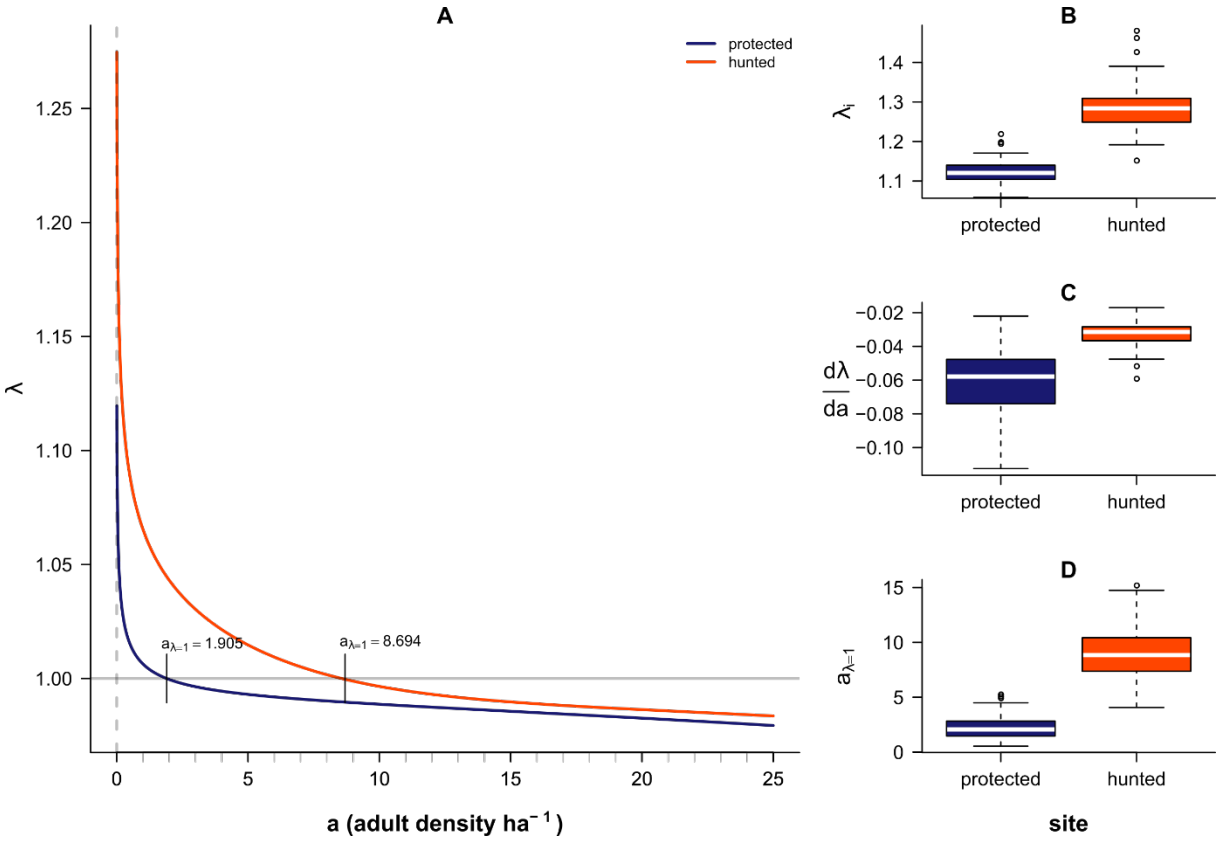


Figure 5. Per capita density-dependent population growth rates for Attalea butyracea for a protected and hunted site in Panama A). The growth rate at low density is the invasion growth rate ($\lambda_{N=0}$), estimated for both sites B), the change in population growth over adult density at the point where $\lambda = 1$ is stabilization rate ($\frac{d\lambda}{dN}$) C) and resulting equilibrium density ($\tilde{N}$) at $\lambda = 1$ are given in D). Statistics in B, C and D were bootstrapped 100 times.

This indicates that protected populations tended to be regulated, on average, with doubled the rate of stabilization compared to hunted populations – though the difference was not

statistically significant. The result of a lower invasion growth rate and a higher stabilization at protected site ensured that the local expected equilibrium density, i.e. the density at which the population is stable (Ñ), was estimated to be 4.5 times lower at protected populations (1.9 trees per hectare; CI 0.92-3.95) compared to the hunted site (8.7 trees per hectare; CI 5.21-12.75; Fig. 5).

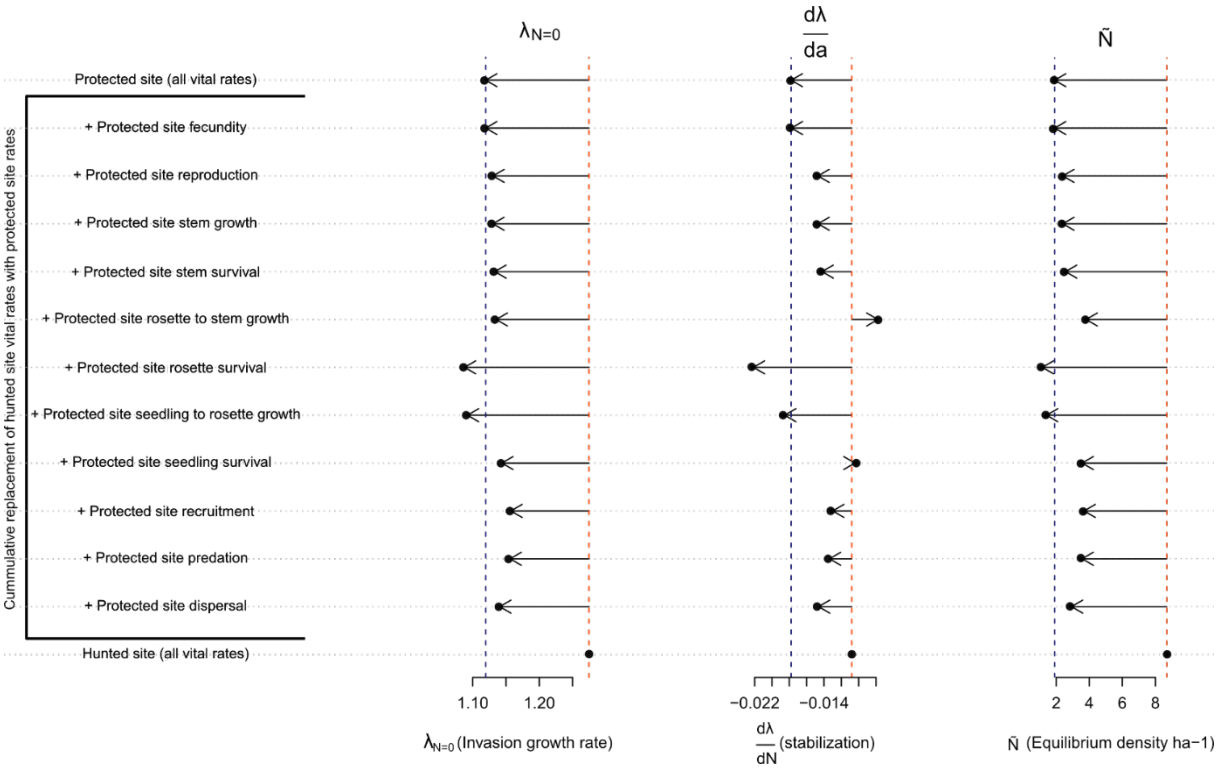


Figure 6. Cumulative influence of individual vital rates across the life-cycle of Attalea butyracea in explaining site differences in invasion growth rates ($\boldsymbol{\lambda_{N=0}}$), stabilization rates ($\frac{\mathbf{d\lambda}}{\mathbf{dN}}$) and resulting expected local densities (Ñ) at a protected and hunted site in Panama. At the bottom row of each panel from left to right $\boldsymbol{\lambda_{N=0}}$, $\frac{\mathbf{d\lambda}}{\mathbf{dN}}$ and Ñ are shown for the hunted and the protected site as modeled from the empirical data. All single vital rates of the hunted site are replaced in order, i.e. from dispersal to fecundity, with the same protected site vital rate. The top row shows the situation of the protected site.

Individual vital rate contributions to site differences

The cumulative replacement of individual subsequent vital rates from hunted sites with rates from the protected revealed the most influential factors that contributed to site differences. Site differences in dispersal were most influential in causing the greater invasion growth rates ($\lambda_{N=0}$) at hunted sites (Fig. 6). Increased seedling to rosette probabilities at hunted sites also contributed to a greater $\lambda_{N=0}$ however this effect appears to be offset by the lower rosette-to-stem probabilities at hunted sites. The estimated weaker rate of stabilization ($\frac{d\lambda}{dN}$) at the hunted site appeared to be partly caused by lower dispersal and partly due to the increased stem survival (Fig. 6). Finally, the vital rate contribution to site differences in expected abundances (Ñ) was influences by all the previously mentioned rates, though site differences in dispersal

again appeared to be the most influential. This is because dispersal is influential on both stabilization and the invasion growth rate. Vital rate contributions to differences in Ñ at protected and hunted sites generally mirrored those for $\lambda_{N=0}$ showing that effect on the invasion growth rate were the most influential in this system.

Discussion

This study theoretically explored how hunting impacts the abundances of plants with multiple life-stages at the population level. We introduce a simple theoretical model predicting that hunting may affect plant abundance by its relative impact on the height of the invasion growth rate and the strength of stabilization (the strength of negative density dependence of the population growth rate). Critical to this framework is understanding how hunting effects seed dispersal, natural enemy attack and interactions with all other vital rates throughout the life-cycle of the focal plants species (Fig 1). We used our model to generate theoretical predictions which we assessed in a case study of a common palm species.

In the case study we quantified the effects of hunting on seed dispersal (Fig. 2), seed predation (Fig. 3), general life history (Fig. 4) and the consequent impact on density-dependent population-level growth rates (Fig. 5). Our results show that hunting increased the projected invasion growth rate and decreased stabilization mainly through its impact on seed dispersal (Fig. 6). Contrary to our expectations (Scenario H2, Fig. 1C), and those generally expressed in the literature (Freckleton & Lewis 2006; Terborgh *et al.* 2008; Bello *et al.* 2015; Osuri *et al.* 2016; Peres *et al.* 2016), we found that the lowered dispersal and strongly increased local seed and seedling densities were not fully compensated by increased negative density-dependence in seed predation (Fig 3), seed recruitment (Fig 4) and seedling survival (Fig 4) resulting in projected equilibrium densities of almost five times greater.

Hunting and dispersal

Average dispersal distances were shown to be 6 times lower when mammals were hunted, confirming our first hypothesis. Disperser counts are lowered as the direct result of hunting, leading to an increased abundance of seeds per disperser and satiation of the remaining dispersers before a substantial part of the fruit crop is removed (Jansen *et al.* 2004; Klinger & Rejmanek 2009).This satiation effect is increased at higher densities of adult palms, leading to undercompensated negative density dependence dispersal (Jansen *et al.* 2014) as we see in Figure 2.

In our theoretical model, the effect of hunting on dispersal is captured by a decrease in parameter *e* (eqn. 1), which leads to an increase in local seed densities as less seeds emigrate to areas outside the population (scenario H1 in Fig. 1). Among the direct impacts of hunting we

documented strongly decreasing seed dispersal distances (Fig. 2), leading to a 620% increase in local seed densities (Fig. 2). All else being equal, we then predicted an increased invasion growth rates for the local population. After integrating all vital rates into a population model, we found increased invasion growth rates (Fig. 5) which was mainly caused by differences in dispersal (i.e. increased local seed densities; see Fig. 6), confirming hypothesis 3a. Our results therefore show that loss of dispersers may in fact lead to increased invasive strength for animal dispersed plant species. This contrasts with the idea that loss of dispersers will always be detrimental for zoochorous species due to increased natural enemy attack close to parents (e.g. Janzen 1970; Freckleton & Lewis 2006; Bagchi *et al.* 2010).

On longer time scales, however, loss of dispersers and decreasing emigrations rates of seeds may still prove to be detrimental to long-term plant fitness. For instance, the rate at which seeds disperse beyond the local population is a major factor influencing the speed at which species spread (Neubert & Caswell 2000). Dramatic climatic shifts are relatively common occurrences (e.g. Stahle *et al.* 2011; Cook *et al.* 2016), and the speed at which species spread will determine their range and whether they reach climate refugia (e.g. Hamrick 2004; Svenning & Skov 2007) when such climate shifts happen. Limited seed dispersal can also be expected to have additional negative long-term effects, such as a reduction of genetic exchange among individuals (Perez-Mendez *et al.* 2016). Therefore, in a completely defaunated world some plants species may become locally dominant, and appear to benefit on short-time scales, but are likely doomed to extinction on longer time scales.

Regardless of the long-term consequences, it is exactly the short-term impacts of hunting that are relevant to conservation and management of natural areas today. To predict ecosystem functioning and emergent properties as carbon storage, it is important to know which plant species will become locally dominant, and whether these dominant species cause competitive exclusion and reduced local biodiversity in the short-term. Natural areas are already highly fragmented and much of earth's biodiversity is increasingly dependent on these fragments (Pimm & Brooks 2013). Here, local dynamics determine the consequences for biodiversity and models of local population dynamics are therefore especially relevant. In such fragmented and isolated communities, highly competitive species, as *Attalea*, are known to locally dominate stands and exclude other species when dispersers are lost (Wright & Duber 2001). To this end, our simple theoretical model can be highly insightful, as our case study clearly demonstrates.

Hunting and natural enemies

Our theoretical model predicts that a critical component in determining local plant abundances is how hunting influences interactions with natural enemies as a response to changes in seed dispersal. Does hunting aggravate enemy attack rates by increasing local densities of seeds and seedlings or alternatively, alleviate natural enemy attack through loss of

vertebrate seed predators? Decay of dispersal services inevitably leads to greater accumulations of seeds in close proximity to parent trees (e.g. Fig. 2). Such seed aggregation is generally expected to limit germination opportunities (Nathan & Muller-Landau 2000) and induce enhanced density dependent seed mortality whenever herbivores, predators and pathogens concentrating around high aggregations of individuals (e.g. Janzen 1970; Comita *et al.* 2014).

Here, we found increased seed mortality rates due to predation (Fig. 3), stronger negative density-dependent seedling survival (Fig. 4B) but similar density-dependent recruitment rates (Fig. 4A). Overall, however, the increased seed predation by host-specialized bruchids and stronger negative density-dependent seedling survival together confirm our second hypothesis. Both these factors correspond to lower values of parameter K in our theoretical model (Fig. 1). Nevertheless, we did not find a stronger rate of stabilization, the integrated negative density dependent rate of stabilization (contradicting hypothesis 3b). Hence, the increased invasion growth rate was not compensated by increased stabilization, leading to a higher projected local abundance (Fig. 5) in opposition to hypothesis 3c. Why did the increased seed predation and negative density dependence not result in greater stabilization? The answer rests on two components: 1) the increased impact of natural enemies must at least compensate for the increased seed densities and 2) stabilization is a population level metric that scales with and depends on all density-dependent and independent vital rates across the life-cycle (see Fig. 1 and below).

Seed predation by bruchid beetles and scatter hoarding rodents showed opposing trends. Attacks by rodents, normally the most important seed predators (i.e. consuming 74% Fig 3; Peguero *et al.* 2017), were reduced by hunting to only 8% of protected site attack rates. Bruchids, in constast, are known to concentrate in seed-rich areas (Janzen 1980) and we indeed found a dramatic increase with up to 82% of the seeds with bruchid beetle scarring. The resulting increase in overall mortality, was however only 8% larger than the protected site. The decline in rodent predation was therefore only marginally compensated by bruchids, and nowhere near to the extent needed to compensate for a 620% increase in seed densities (Fig. 2). Recruitment from seed to seedling was indistinguishable between sites, and therefore the increased recruit densities are the direct result of defaunation. These monospecific aggregations of seedlings around conspecific palms, however, did induce stronger density-dependent seedling mortality (Fig. 4). This increase in negative density-dependent seedling mortality, most likely soil-borne pathogens induced (Jansen *et al.* 2014), only reduced survival by ± 8% (a drop from approximately 70% to 62% at most; Fig 4). To fully compensate for the 620% increase in seed densities, the combined effect of seed predation and seedling survival should have been 5-fold stronger. To our knowledge, no other study has documented whether increases in the mortality of seeds and seedling in hunted sites compensates for the loss of

dispersers. Hence there still appears to be a general lack of knowledge on compensation, as was already mentioned by Freckleton & Lewis (2006), with this being the first study to fully contradict it. We must, therefore, conclude that the often stated claim (Janzen 1970; Terborgh *et al.* 2008; Bagchi *et al.* 2010; Bello *et al.* 2015; Osuri *et al.* 2016; Peres *et al.* 2016) that negative density-dependence will synergistically interact with the loss of dispersers to limit local abundance of plant species to be unsubstantiated.

Interactions with higher life stages

It is obvious that all vital rates contribute to population dynamics of forest trees. Predicting how vital rates contribute, however, is more far challenging (see e.g.Visser *et al.* 2016). The theoretical framework presented here predicts that the invasion growth rate and stabilization together determine plant abundance and that both these metrics are scaled by vital rates across the life-cycle. The invasion growth rate is the per capita increase of the population at low density, and the effects of vital rates such as the maturation rate of juveniles, adult survival and seed production are straightforward to predict: increases in any of the former rates lead to concurrent increases in the invasion growth rate (Fig. 1, Fig. S4). Any conspecific density-dependent factor (*K*) has a negligible effect when the focal species' density goes towards zero, and therefore by definition has no influence on the invasion growth rate. Population growth rates at low densities depends on factors other than conspecific density, depending, for instance, far more on adaption to the local environment or the combined effects of competitors (Chesson 2000).

Stabilization is the decrease in the per-capita population growth rate with increasing density of the focal species, and here, the effects of different vital rates interact in more complex ways (Fig. 6). Nevertheless, their effect on stabilization can be intuitively understood. For instance, greater adult survival rates or maturation rates - all else being equal - leads to stronger stabilization (Fig. S4). This is because increases in both rates lead to more adults in the population, which means increases in the total number of seed produced, and finally stronger net density dependent mortality – whenever the net recruitment rate is a function of the local seed density (as in our model; Fig. 1). Hence, two sites can be identical in all aspects (e,m,r and K) other than adult survival (s) and have completely different apparent density-dependent recruitment, invasion growth and stabilization rates. The final realized plant abundance then depends on how much the invasion growth rate is increased relative to the strength of stabilization (Fig. 1). Our simple model underscores that it is of paramount important to correct for difference in all vital rates between sites, before fair comparisons can be made. We discuss the consequences of this for our case study in the next section.

Interactions with site effect other than hunting

In our case study, the projected invasion growth and stabilization rate were influenced by later life-stages and vital rates in addition to dispersal and seedling survival. Could it be that the projected increase in plant abundance in the hunted site are, at least in part, due to factors unrelated to hunting? And if so, how important are the relative effects of hunting compared to various site specific effects? Fortunately, our applied methodology allows us to distinguish the degree to which effects – those that can be plausibly assigned to hunting, and those that cannot - are responsible for the observed difference in population dynamics (shown in Fig. 5).

We start from the premise that both the increased seed and seedling densities are the direct result of loss of dispersers. Our reasoning follows three steps. First, seed production rates (Fig 2) do not differ significantly between sites, and this leaves only the differences in dispersal to explain the increased seed densities between sites (at equal adult abundances). Second, seedling recruitment rates are almost identical between sites (Fig. 4A), and hence the hunted site must have greater absolute numbers of seedling recruits due to greater local seed densities (Fig. 2, 4C). Finally, we observed an increased density-dependent mortality of seedlings (Fig. 4B) which can, in turn, be attributed to greater seedling densities due to the previously explained recruitment. All other site difference are less directly linked to defaunation, and may be caused by unrelated biotic or abiotic difference between sites. Therefore, we conservatively assume these to be unrelated to hunting.

The invasion growth rate was most strongly affected by the site-wide decrease in dispersal in the hunted forest (Fig 6), i.e. the largest change in the invasion growth rate is caused by the 620% increase in local seed densities. It was also, for a smaller part, affected by lower seedling to rosette and higher rosette to stem transitions in hunted sites. Stabilization was influenced by a more complex interplay of vital rates, most noticeably by higher seedling to rosette growth and lower rosette to stem growth at the hunted site (Fig 7). It appears, that local site difference – other than hunting – could have influenced site differences in the strength of stabilization. Nevertheless, the differences in stabilization between sites are minimal and not significant. This is emphasized by the fact that the projected equilibrium densities are far more sensitive changes in the invasion growth rate. Showing that effects on the invasion growth rate are critical, at least in our case study, and here the effects of hunting were overwhelmingly more important.

General applicability

Our theoretical predictions concerning the population-level effects of dispersal, should also be generally applicable. Declining animal seed dispersal rates by defaunation has been documented in a multitude of different studies (Wright *et al.* 2000; Beckman & Muller-Landau

2007; Donatti *et al.* 2009; Rosin & Poulsen 2016). In this previous work seed removal rates were 4.32 (± 1.65 SE) times greater in protected sites compared to hunted sites. Simulations of disperser loss are also predicted to lead to similar increases in aggregation as reported here (e.g. 4 fold; Caughlin *et al.* 2015). Defaunation always lowers seed removal rates in animal dispersed species, which always increases the proportion of seed remaining in the population (i.e. a decreasing *e* in eqn 1). This will lead to increased local seed densities which – all else being equal – will increase invasion growth rates of the focal species. Decreasing seed removal rates also will inevitably lead to greater aggregation of seeds, and therefore the question is whenever hunting also affects additional vital rates across the life cycle. In that case, the net effect of on both the invasion growth rate and stabilization should be estimated.

Past work has found generally positive effects of defaunation on seed predation, recruitment rates and seedling densities (Wright *et al.* 2000; Beckman & Muller-Landau 2007; Dirzo *et al.* 2007; Wright *et al.* 2007b; Donatti *et al.* 2009; Vanthomme *et al.* 2010; Effiom *et al.* 2013; Rosin & Poulsen 2016; Granados *et al.* 2017). Seed mortality, on average was 2.01 (± 0.83 SE) times greater in protected sites, recruitment and germination 1.86 times (± 0.97 SE) higher in defaunated sites, and seedling densities tended to be 1.59 (± 1.71 SE) times larger in defaunated sites as well on average as well. It therefore certainly appears that natural enemies cannot compensate for increased seed densities in hunted site or that the most important natural enemies are also killed by hunters. Unfortunately, as no other study has empirically shown whether compensation takes place at the population level we cannot be completely certain. Theoretically we can predict, however, that this should lead to increased invasion growth, decreased stabilization rates and local dominance of animal dispersed species. Given our model, and current evidence, we must therefore cautiously conclude that there is no evidence for widely assumed demise of animal dispersed species - at shorter time scales at least. We can be certain, however, that hunting does influence (density-dependent) plant-enemy interactions and based on previous work, and our own, this tends to lower mortality rates and increase regeneration. Our work therefore provides a clear warning that dynamics measured in intact forests may not be representative to simulate the consequences of hunting (Caughlin *et al.* 2015)– and hence such results should be interpreted with caution.

Conclusions

This was the first study to integrate all effects of hunting across the entire life cycle of a common species in both a protected and hunted site. Our empirical results fitted our theoretical framework, and shows that we may generally expect hunting to lead to increases in low density population growth rates due to greater seed densities in the local population. We also expect hunting to reduce seed predation rates, and aggravate natural enemy attack rates at later juvenile stages and thus - - given results documented both here and elsewhere - natural

enemies of plants are unlikely to fully compensate for the loss of dispersers. As a result, the dire predictions of loss of large-seeded animal-dispersed species, and consequent effects on carbon stocks, may be premature. As shown here, loss of animal dispersers could lead to local dominance of animal dispersed species – at least on the short term. Especially when animal dispersed species are superior competitors, or can be assumed to be, as is the case with the hyperabundant palm *Attalea butyracea* (ter Steege *et al.* 2013) - this may lead to competitive exclusion and have negative consequences for local biodiversity.

It is clear from our study, and previous work, that hunting alters plant-animal interactions. Because plant-animal interactions affects multiple life stages, the effect on the population of these losses are non-trivial to predict and have non-intuitive consequences. We confirm this with both a simple theoretical model (Fig 1) and with well-studied and relatively simple model system as *Attalea* (Fig 5, 6). We believe that our work unequivocally shows that one simply cannot predict ecosystem, community or even population-level effects of hunting from single life stage studies or simulative studies done in intact forests (Arroyo-Rodriguez *et al.* 2009; Bello *et al.* 2015; Caughlin *et al.* 2015; Osuri *et al.* 2016; Peres *et al.* 2016). To fully understand the effects of hunting full life-cycle studies on many more species are urgently needed, and this requires long-term measuring efforts of populations and communities as a whole.

Our results stress that investigations into the entire life-history, like this one, are crucial before we can even hope to start predicting how species dynamics will change with anthropogenic disturbances (Beckman *et al.* 2012). Here, we provide a theoretical framework with which to evaluate the effects of hunting on local plant abundance. We tested this framework with a case study showing its empirical feasibility and relevance.

Acknowledgements

The authors would like to thank Gemma Rutten, Jasper Ruifrok and Pablo Ramos for their help in the field.

Supplementary materials

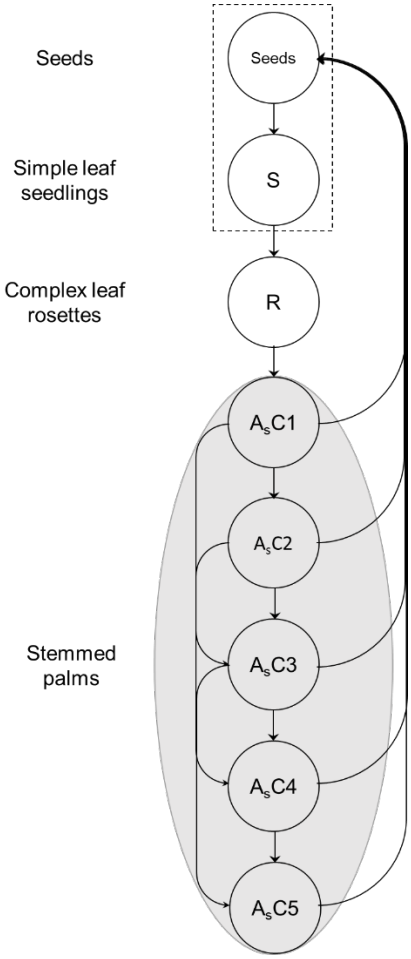


Figure S1. Schematic of the life cycle of Attalea butyracea and the transitions (arrows) between the key life stages. Key life stages are seeds and seedlings(S) together, rosettes (R), and stemmed palms (A)). Adult reproductive rates were dependent on crown position (C1-C5).

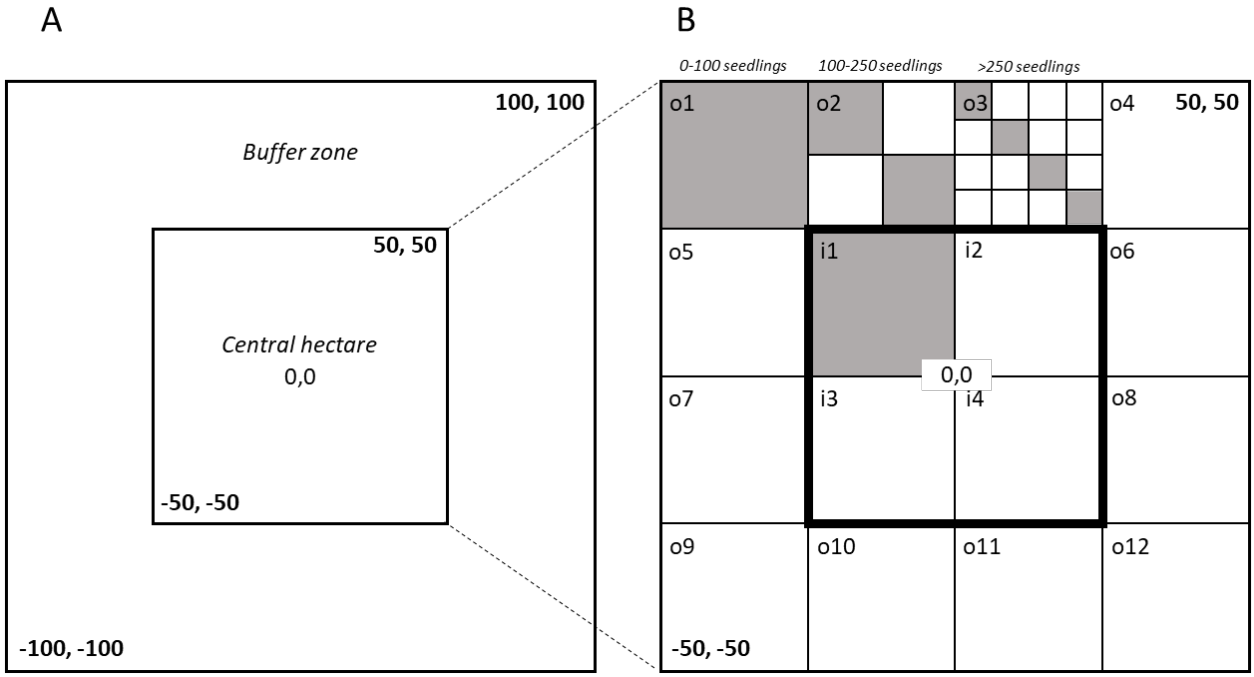


Figure S2. Plot lay-out. A) All adults were censused in the 200 x 200 m. plots. B) Seedlings were only measured within the central hectare which was divided into 16 25 x25m subplots. There were 4 subplots in the inner ring (i1-i4) and 12 subplots in the outer ring (o1-o12). In each plot 4 subplots were randomly assigned for the seedling census: 1 in the inner ring and 4 in the outer ring. When there were between 100 and 250 seedlings within a subplot, halve of the subplot was censused in two diagonal subquadrates of 12.5x12.5m and when there were more than 250 seedlings in a subplot a quarter of the subplot was censused in 4 diagonal subquadrates of 6.25x6.25m each.

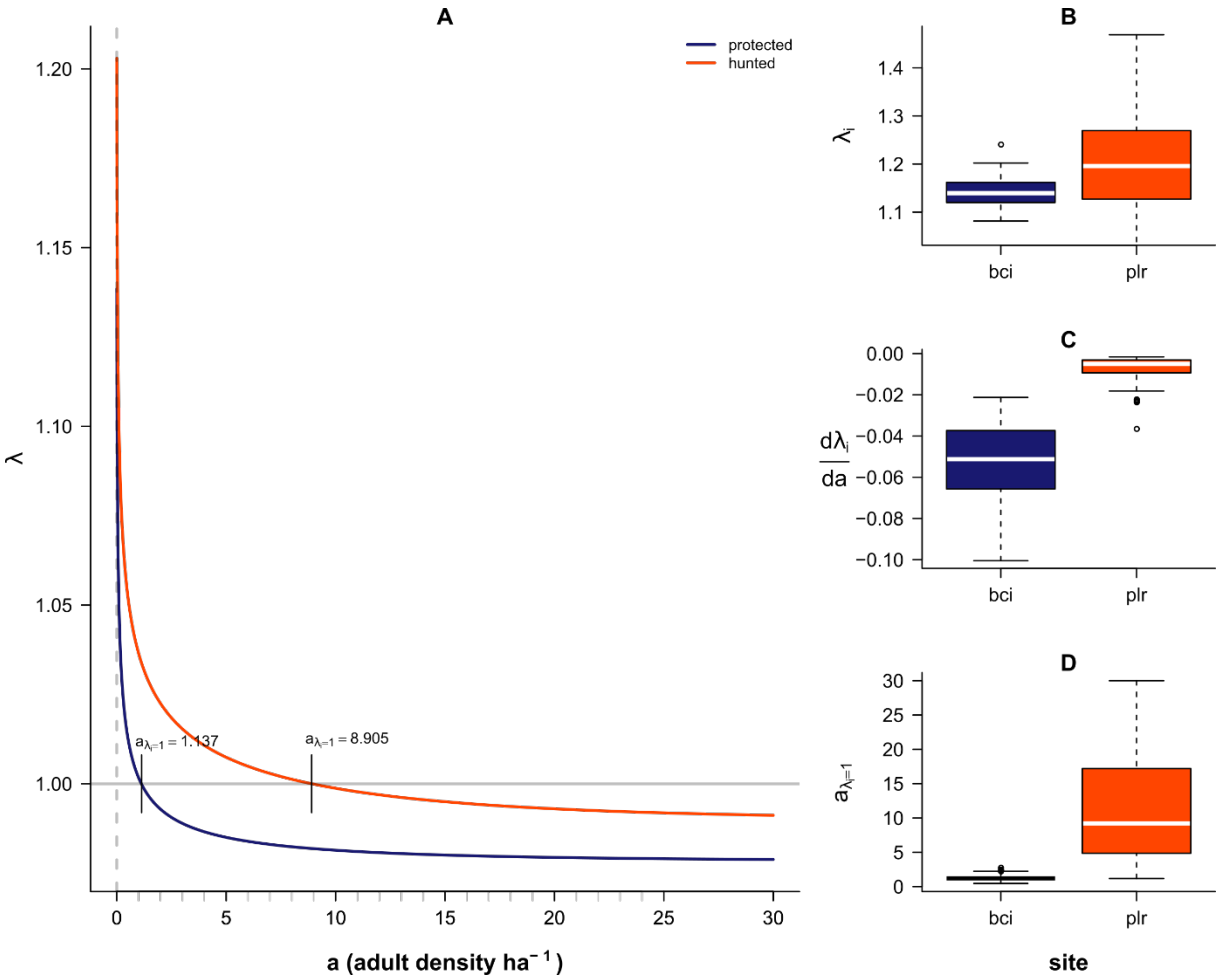


Figure S3. Per capita density-dependent population growth rates for Attalea butyracea for a protected and hunted site using only the best model fits. A). The growth rate at low density is the invasion growth rate ($\lambda_{N=0}$), estimated for both sites B), the change in population growth over adult density at the point where $\lambda = 1$ is stabilization rate ($\frac{d\lambda}{dN}$) C) and resulting equilibrium density ($\tilde{N}$) at $\lambda = 1$ are given in D). Statistics in B, C and D were bootstrapped 100 times.

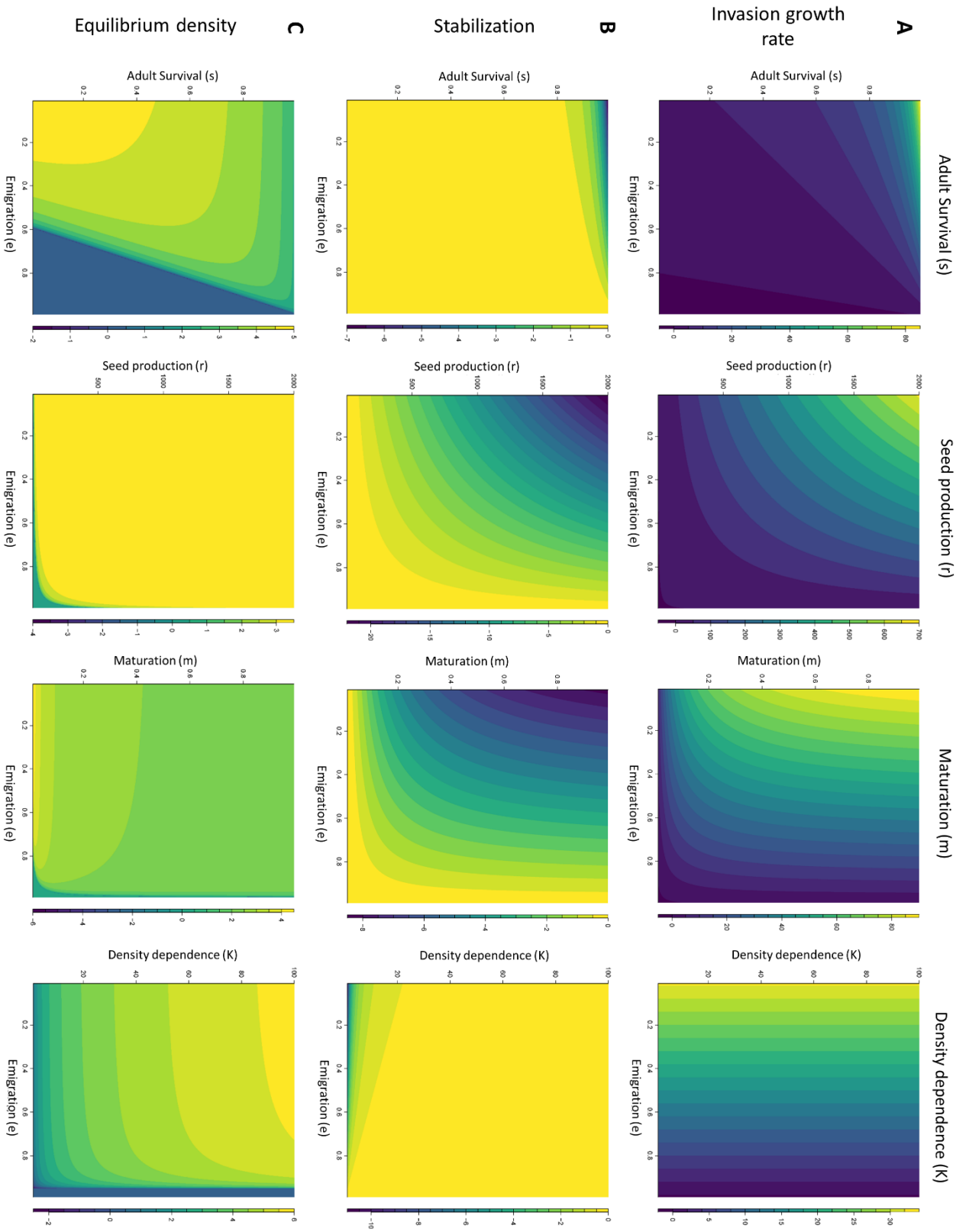


Figure S4: Model behavior in response to seed emigration (e) and the other parameters (i.e. m,r,s,K). A) Expected invasion growth rates increase with higher maturation and fecundity and is independent of K. B) A lower K will strengthen stabilization as well as higher survival, seed production and fecundity. C) The expected abundances increase with adult survival and K and lowered by e.

Table S1. Density-dependent functions of vital rates of the palm Attalea butyracea. With averaged model coefficients under site- and/or density-dependence. Bold face indicates significance. In each function, parameter β0 is the intercept, β1 is the density dependent effect, and β2 is hunted effect

Variable	Estimate ± SE				Form
	Protected (intercept)	Hunted (intercept)	Density	Interaction (density x hunted))	
Fecundity	6.96±0.57	7.14±0.51	-0.23± 0.3	NaN	log(fec)~log(dens)+site
Mean dispersal distance	4.31±0.4	2.58±0.35	-0.5±0.21	NaN	log(md) ~ log(dens) + site
Seed densities	-0.85±0.38	1.12±0.34	1.2±0.2	NaN	log(sdens) ~ log(dens) + site
Rodent Predation rates	1.067 ± 0.12	-2.69 ± 0.31	-0.003 ± 0.007	-0.077±0.025	SF~Logit^{-1}((dens) * site)
Bruchid Predation rates	-1.055± 0.11	0 1.442± 0.13	0.039±0.006	0.012±0.009	SF~Logit^{-1}((dens) * site)
Total Predation rates	1.34± 0.13	1.73± 0.15	-0.005±0.007	-0.005±0.010	SF~Logit^{-1}((dens) * site)
Seed to seedling	-3.61±0.26	-3.65±0.18	-0.58±0.18	-0.58±1.28	c(rd/sd) ~ log(dens) * site, weights = area/2500)
Seedling survival	1.05±0.13	1.41±0.19	0.007±0.008	-0.09±0.02	SF ~ Logit^{-1}(dens * site + (1 | census))
Seedling to rosette	-4.06±0.53	2.73**±**0.75	0.018**±**0.03	-0.019**±**0.07	SF ~ Logit^{-1} (dens * site + (1 | census) + (1 | plot))
Rosette survival	2.17±0.6	2.32**±**1.24	0.008**±**0.06	0.009**±**0.13	SF ~ Logit^{-1} (dens * site + (1 | census) + (1 | plot))

Variable	Estimate ± SE				Form
	Protected (intercept)	Hunted (intercept)	Density	Interaction (density x hunted))	
Rosette to stem	-4.04±0.56	-5.2±3.74	-0.075±0.06	-0.12±0.63	SF ~ $Logit^{-1}$ (dens * site)
Stem survival	C12: 3.16±0.59 C13: 3.08±0.56 C14: 2.5±0.52 C15: 2.6±0.49	3.39±0.95	-0.021±0.03	-0.023±0.071	s ~ $Logit^{-1}$ (d1 + dens * site + (1 | plot))
Reproduction	C12: 0.42± 0.17 C13: 1.52± 0.2 C14: 2.26± 0.31 C15: 2.01± 0.31	-0.72±0.61	0.044±0.028	0.039±0.061	s ~ $Logit^{-1}$ (d1 + dens * site + (1 | plot) + (1 | census))

Table S2: Equations for estimating transition values in matrix A. All functions were fit to data.

Construction of matrix A(a,z)

Kernel	Formulation	Description
$H_i(a,z,c)$	$= \begin{cases} S_i(a,z) - \sum_{j \neq i} S_i(a,z) T_{i \to j}(a,z) & i \leq 2 \\ S_i(a,z,c) - \sum_{j \neq i} S_i(a,z,c) T_{i \to j}(a,z) & i > 2 \end{cases}$	$S_i(a,z)$ and $T_{i \to j}(a,z)$ are the density and site dependent survival for class i and transition rates from i to class j.
$G_{i \to j}(a,z)$	$= S_i(a,z) T_{i \to j}(a,z)$	Models as described above. $G_{i \to j}(a,z)$ is defined as zero where no transitions are allowed see eqn. X in the main text.
$B_{i \to j}(a,z,c)$	$= \begin{cases} F(a,z) P(a,z) E(a,z) R(a,z,c) S_i(a,z,c) & i \geq 3 \\ 0 & i < 3 \end{cases}$	Models $F(a,z,m)$, $P(a,z)$, $R(a,z,c)$ and $E(a,z)$ are the density and site dependent rates of seed production, seed predation and probaility of reproduction and the seed to seedling transition probabilities. Model F is also a function of the median seed dispersal distance. .

Table S3. Sample sizes for adults and seedlings per census.

	2008		2010		2012	
	Protected	Hunted	Protected	Hunted	Protected	Hunted
Seedlings	601	855	607	590	646	1116
Rosettes	341	670	339	639	341	721
Adults	407	401	NaN	NaN	403	597

*Table S4: Transition probabilities crown positions and model results. Model format: c2 ~ c1 + density * site*

	Estimate	Std. Error	z value	p-value
2(protected)	1.545	0.551	2.804	**
2(c12)	1.078	0.53	2.034	*
2(c13)	0.512	0.454	1.127	ns
2(c14)	0.255	0.68	0.375	ns
2(c15)	1.279	0.777	1.647	ns
2(density)	-0.028	0.031	0.897	ns
2(hunted)	0.249	0.435	0.573	ns
3((protected))	-1.201	0.641	1.873	ns
3(c12)	1.957	0.562	3.481	***
3(c13)	1.800	0.482	3.731	***
3(c14)	1.977	0.683	2.896	**
3(c15)	2.499	0.790	3.128	**
3(cens)	0.039	0.033	1.173	ns
3(hunted)	1.511	0.482	3.137	**
4(protected)	-1.20	0.687	1.753	ns
4(c12)	1.342	0.613	2.189	*
4(c13)	0.930	0.543	1.712	ns
4(c14)	2.319	0.696	3.331	***

	Estimate	Std. Error	z value	p-value
4(c15)	3.184	0.803	3.966	***
4(density)	0.038	0.036	1.072	ns
4(hunted)	0.871	0.522	1.668	ns
5(protected)	-5.032	1.252	4.02	***
5(c12)	2.056	0.893	2.303	*
5(c13)	1.413	0.877	1.611	ns
5(c14)	1.056	1.318	0.802	ns
5(c15)	4.193	0.99	4.235	***
5(density)	0.136	0.056	2.436	*
5(hunted)	1.818	0.784	2.317	*
2(density:hunted)	0.004	0.011	0.43	ns
3(density:hunted)	0.006	0.013	0.461	ns
4(density:hunted)	0.008	0.017	0.48	ns
5(density:hunted)	0.011	0.023	0.481	ns

(conditional average)	Estimate	Std. Error	z value	p-value
2(protected)	1.545	0.551	2.804	**
2(c12)	1.0775	0.53	2.034	*
2(c13)	0.512	0.454	1.127	ns
2(c14)	0.255	0.68	0.375	ns
2(c15)	1.279	0.777	1.647	ns
2(density)	-0.028	0.031	0.897	ns
2(hunted)	0.249	0.435	0.573	ns
3(protected)	-1.201	0.641	1.873	ns
3(c12)	1.957	0.562	3.481	***
3(c13)	1.800	0.482	3.731	***
3(c14)	1.977	0.683	2.896	**

	Estimate	Std. Error	z value	p-value
3(c15)	2.499	0.799	3.128	**
3(density)	0.039	0.033	1.174	ns
3(hunted)	1.511	0.482	3.137	**
4(protected)	-1.204	0.687	1.753	ns
4(c12)	1.342	0.613	2.189	*
4(c13)	0.93	0.543	1.712	ns
4(c14)	2.319	0.696	3.331	***
4(c15)	3.184	0.803	3.966	***
4(density)	0.038	0.036	1.073	ns
4(hunted)	0.871	0.522	1.668	ns
5(protected)	-5.033	1.252	4.02	***
5(c12)	2.056	0.893	2.303	*
5(c13)	1.414	0.877	1.611	ns
5(c14)	1.056	1.318	0.802	ns
5(c15)	4.193	0.99	4.235	***
5(density)	0.136	0.056	2.439	*
5(hunted)	1.817	0.784	2.317	*
2(density:hunted)	0.073	0.076	0.972	ns
3(density:hunted)	0.097	0.078	1.241	ns
4(density:hunted)	0.124	0.082	1.511	ns
5(density:hunted)	0.175	0.114	1.531	ns

Relative variable importance:

	d1	site	density	density:site
Importance:	1.00	1.00	1.00	0.06
N containing models:	5	3	3	1

6. Synthesis

Vervang door opgemaakte versie

Species influence each other in many complicated ways in their pursuit for resources and the struggle for existence. Together these species and their interactions shape the dynamics and the structure of communities and ecosystems as we know them. Classically, studies on these interactions, such as predator-prey interactions, are mostly centered around trophic interactions, with food webs as paradigmatic examples of ecological networks (Pimm 1979; Allesina *et al.* 2008). Over the last decades, however, an increasing number of studies revealed that non-trophic interactions may also play an important role in shaping species interactions and even entire ecosystems (Bruno *et al.* 2003; Hastings *et al.* 2007). Therefore, many of these studies call for an integration of trophic and non-trophic networks (Olff *et al.* 2009; Kefi *et al.* 2012). However, although gaining interest, the integration of trophic and non-trophic interactions into a single framework has been mostly theoretical until now. Very little empirical, let alone experimental, work has been carried out on this topic. Foundation species, spatially dominant species that have a strong community-structuring role, are predominantly described by their non-trophic effects such as habitat-modification and stress mitigation. However, even in these systems, and although their proposed non-trophic effects on the food web have often been asserted, studies are only recently trying to disentangle their trophic effects from their non-trophic effects and even and even those have remained correlative or theoretical in nature (Miller *et al.* 2015; van der Zee *et al.* 2016). Furthermore, foundation species' effects are often studied in a unidirectional way, in which the reciprocal effect of the facilitated species on its foundation species is often excluded.

In this thesis, I have taken the first steps towards separating trophic and non-trophic effect of interspecific interactions of foundation species and their communities, and additionally zoomed in on single interactions with facilitated species. I aimed to 1) assess the effect of foundation species on the food web, and 2) disentangle the roles of trophic and non-trophic roles of foundation species and their associated community, and (3) investigate to what extent such interactions are reciprocal in nature.

To answer these main questions, I first defined the interactions between foundation species and their associated community in a trophic and non-trophic context (Chapter 1). Next, I showed how foundation species affect emergent properties of the local food web and the community in a wide range of ecosystems (Chapter 2; Fig. 1). To further understand the relative contribution of trophic versus non-trophic interactions by foundation species on their communities, I experimentally studied how the iconic foundation species Spanish moss, in one of the ecosystems addressed in Chapter 2, affected its resident invertebrate community (Chapter 3; Fig. 1). The interactions between foundation species and their resident communities are often thought of as mostly unidirectional interactions. Recent work, however, suggests that foundation species may also engage in mutualistic interactions with associated community members (e.g. Stachowicz 2001; van der Heide *et al.* 2012; Angelini *et al.* 2016),

similar to earlier studies showing that plant roots and their associated microbiome (including mycorrhizae and nitrogen fixing bacteria), are also engaged in tight mutualisms (van der Heijden *et al.* 1998; DeLuca *et al.* 2002). To understand how Spanish moss interacts with its primary foundation species, *Quercus virginiana*, I studied the interactions between this primary facilitating species and its facilitated species, the secondary foundation species Spanish moss, and with the species facilitated by the secondary foundation species itself (Chapter 4; Fig. 1). Finally, I empirically tested the effect of the removal of a mutualistic species in a model system of neotropical palms and their rodent dispersers to better understand the population-level effects of the facilitated species on its foundation species (Chapter 5; Fig. 1).

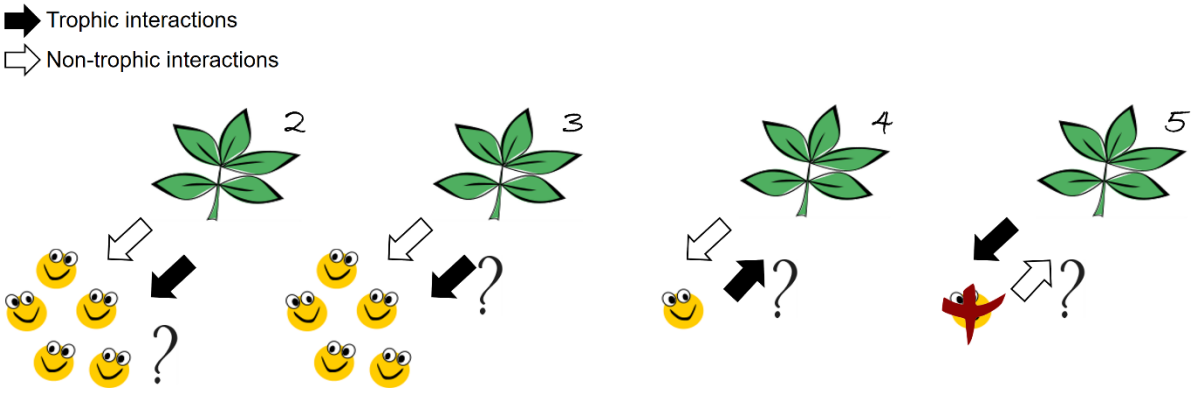


Figure 1: Simplified graphical representation of research questions addressed in the chapters of this thesis (represented by numbers). In Chapter 2, I studied the effect of different foundation species on their facilitated food webs. In Chapter 3 I experimentally studied whether these effects were trophic or non-trophic. In Chapter 4, I studied the long-term trophic effect of a facilitated species on its foundation species. In Chapter 5, I experimentally studied the contribution of a trophic versus a non-trophic interaction in a seed-disperser interaction.

Foundation species facilitate food webs across trophic levels

Although food webs are an important characteristic of ecosystems, the mechanisms shaping these complex networks remain largely unknown (Allesina *et al.* 2008; Thompson *et al.* 2012; Baiser *et al.* 2013; Monteiro & Faria 2016). Foundation species are generally thought to increase ecosystem biodiversity by habitat modification and stress mitigation (Bertness & Callaway 1994; Bruno & Bertness 2001; Bruno *et al.* 2003). Recently, these non-trophic species interactions such as habitat modification and mutualisms have been suggested to be important determinants of food web structure (Baiser *et al.* 2013; Angelini & Silliman 2014; van der Zee *et al.* 2016). However, it remains unclear whether these findings generalize across different ecosystems, and whether non-trophic interactions affect specific trophic levels or functional groups such as predators or herbivores. I have therefore carried out an in-depth analysis of the effects of foundation species on the food web characteristics in 7 different ecosystems ranging from terrestrial to coastal and freshwater systems (Chapter 2).

We found food web size and complexity (i.e. link density) were invariably enhanced by the presence of foundation species across all ecosystems. An analysis of 19 measured food web metrics showed pervasive modifications in food web characteristics in foundation species-dominated food webs compared to bare food webs. Although the trophic network was drastically enhanced by the presence of foundation species, we only found few trophic links connected to the foundation species themselves. This also indicated that foundation species were not being used as a food source by most species. Interestingly, this low connectedness of the foundation species, also found in earlier work (van der Zee *et al.* 2016), suggests that non-trophic effects, such as habitat modification and stress mitigation, were the main drivers of the modifications in the food web.

Our analyses further revealed that random removal of nodes from foundation species-dominated food webs produced networks with very similar characteristics as the bare areas, showing that foundation species facilitate species across all trophic levels (also see box 1). However, four metrics – a lower basal species fraction, and a higher carnivore fraction, average chain length and average trophic level – deviated consistently across food webs from the networks where nodes were randomly removed, showing relatively longer food chains in foundation species-dominated food webs than expected with random facilitation. This result suggests a higher level of specialization of the species that are facilitated by foundation species, but it may also be a more general effect when food webs become more complex (box 1).

Box 1: *Random facilitation as a general mechanism*

I have shown in Chapter 2 that random removal of nodes yields food webs with similar characteristics to bare area food webs. The question remains whether these changes are confined to foundation species only. Our hypothesis was that foundation species food webs are larger in size, but structurally similar to other ecological networks. To test this hypothesis, I have carried out additional analyses using the well-known Niche model by Williams and Martinez (2000). The niche model only needs species number and connectance (i.e. satiation of the network) as input parameters, and creates networks similar to real ecological networks by ranking the species randomly and assigning them all a hypothetical 'niche' range. Species consume species that fall within this range, creating a model food web. I first created the same number of replicate niche models per ecosystem as the empirical food webs, using average connectance and species numbers within the 95% confidence interval of foundation species-dominated food webs per system as input parameters. As a second step, I randomly removed nodes from the niche model networks to the species level of the corresponding bare area networks, and did the same for the real networks (for a full explanation on that process see Chapter 2). Next, I calculated 19 often-used food web metrics for all foundation species-dominated, bare area, niche model and random removal niche model networks. A principal

component analysis (PCA) of these metrics per network shows a similar result as shown in Fig. 3 in Chapter 2. The first axis already explains the bulk (96%) of the variation and on this axis the foundation species networks were indistinguishable from the niche model networks in terms of metrics. Furthermore, the bare area networks are not statistically different from niche models with randomly removed nodes (Fig. 1). In addition, the same four metrics, basal species, carnivores, chain length and trophic level seem to deviate the most between niche model and empirical networks. The use of the niche model shows that the modifications by foundation species in the food web seem to be generally applicable to any food web, which seems indicate these modifications would happen in any food web when species are facilitated.

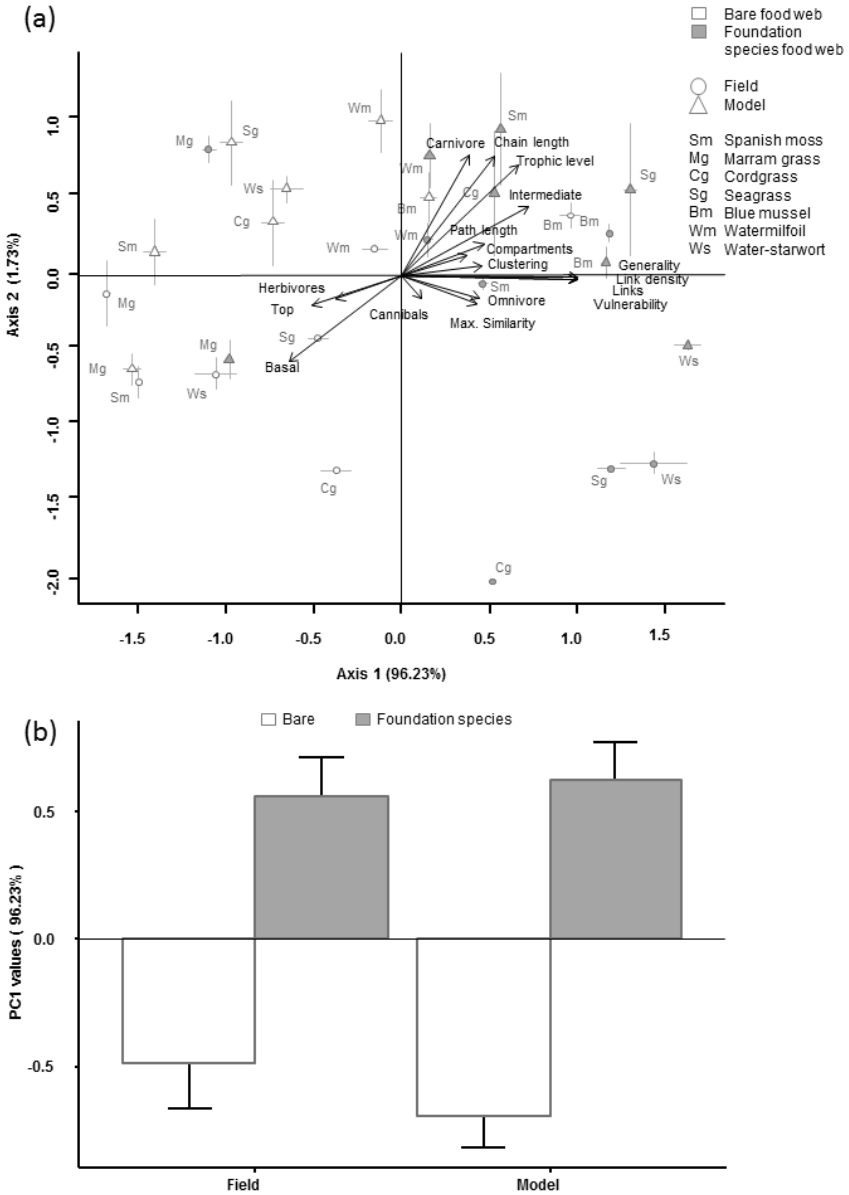


Figure 1. (a) Averaged PCA values (mean±SE) of all food web metrics describing both field and niche modelled networks of foundation species and bare areas. Arrows are projected food web metrics (total variation 1502, axis 1: 96.23%, axis 2: 1.73%). (b) Scores of Principle Component axis 1 explained by bare versus foundation species ($p < 0.0001$), and model versus field data (ns).

Spanish moss as furniture and food for its epifaunal community

Foundation species can enlarge the food web by facilitating the community (Chapter 2) through the increase of habitat structure and mitigation of physical stress (Bruno *et al.* 2003). As I have shown in Chapter 2, foundation species typically show fewer trophic connections compared to other species in the food web. Also, foundation plant species are generally considered to be low-quality food sources for herbivores (Jaschinski *et al.* 2011; Wright *et al.* 2014; Miller *et al.* 2015; van der Zee *et al.* 2016). The trophic contribution of foundation species in facilitating other species is therefore generally considered of minor importance compared to their non-trophic role (van der Zee *et al.* 2016). However, few studies have experimentally unraveled the relative importance of the non-trophic versus the trophic contributions of foundation species as a direct food source (see Wright *et al.* 2014; Kefi *et al.* 2015; Miller *et al.* 2015; van der Zee *et al.* 2016). I here aimed to disentangle the trophic and non-trophic contributions in the epiphytic plant *Tillandsia usnoides* (Hereafter Spanish moss). Spanish moss is a rootless epiphyte found abundantly in the southern coastal plain of the United States. It is a secondary foundation species as it hangs in clumps (festoons) from the branches of the primary foundation species Southern Live Oak (*Quercus virginiana*) (hereafter oak). Spanish moss dramatically increases habitat structure within the oak's canopy, creating habitat for many invertebrate species, and even some mammal species, that are facilitated by its structures (Angelini & Silliman 2014). I chose to use the oak-Spanish moss habitat as a model system for my experiment because Spanish moss lacks a root system or other attachment structures, making its presence and abundance easy to manipulate (Garth 1964). In my experiment I compared living Spanish moss festoons to plastic mimics and to bare branch. I analyzed the invertebrate community that colonized the structures in terms of species richness and composition by distinguishing between feeding guilds. Also, I measured non-trophic effects of both living and mimic festoons, such as their potential dampening effect on temperature fluctuations, their capturing of external particulate matter, and their role as a nursery. Furthermore, I studied the influence of patch size by studying the community of real and mimic festoons in four different size classes.

Non-trophic effects of the living and mimic Spanish moss were similar but enhanced, compared to bare branches. Enhancement of habitat structure alone (i.e. Spanish moss mimics) facilitated the community, as species richness and number of feeding guilds increased compared to bare branch, but real Spanish moss increased species richness even further. Detritivores, scavengers and predator numbers all increased in living Spanish moss compared to mimics. A significant part of the community seemed to trophically depend on Spanish moss as detritivores and scavengers thrived on its detritus, who in turn where predated upon by higher trophic levels. Overall, of the total increase in species richness ~40% was mediated by

structure alone (mimics), but the additional ~60% depended on Spanish moss as a food source. This effect was seemingly independent of festoon size as there were no statistical interactions found. Surprisingly, the detritivores increased the most in living Spanish moss compared to mimic, while herbivores had very low numbers and did not increase in living versus mimic festoons, or with festoon size. This shows that foundation species may indeed have an important role in the food web – not as a direct food source, but more indirectly through their detritus. Normally in food webs studies detritus is often collapsed into a single node in food webs (Moore *et al.* 2004), especially in the so called 'green food webs' that focus on the use of living plant tissue (Chapter 2). The results of our study on Spanish moss indicate the importance to differentiate the sources of detritus. Detritus is a key element in nutrient cycling through the system and overall productivity and may be an important food resource for many species including species associated with other foundation species (Moore *et al.* 2004; Miller & Page 2012).

Facilitated Spanish moss feeds its host tree

From Chapter 2 and 3 I concluded that foundation species have far-reaching and pervasive effects in facilitating the local species community, by providing habitat for many species. Facilitation is an interaction that is generally only unidirectionally defined from the perspective of the facilitated species. Foundation species themselves are also often partners in mutualistic interactions that enhance their own growth (Stachowicz 2001; van der Heide *et al.* 2012; Angelini *et al.* 2016). In Chapter 4, I have investigated the reciprocal interactions between the secondary foundation species Spanish moss and its primary foundation species, Southern live oak.

Epiphyte-host interactions, in our case Spanish moss-oak, are generally presumed to be commensalistic (neutral effect on host) or parasitic (negative effect on host) (Johansson 1974; Blick & Burns 2009; see Chapter 1 Table 1). The tree provides substrate and optimal growing conditions for Spanish moss. Spanish moss, on the other hand, lacks a root system and until now it was unclear what its main source of nutrients is (Garth 1964). There are four possible nitrogen sources Spanish moss can use for its growth: nutrients leaching from the tree (Garth 1964; Benzing & Seemann 1978), while also wet/dry deposition (Nadkarni 1986; Angelini & Silliman 2014), atmospheric nitrogen fixation by associated bacteria (DeLuca *et al.* 2002), or external particulate matter in the form of dust or excrements from its facilitated community (Angelini & Silliman 2014; Chapter 3. In addition, it was unclear whether Spanish moss could play a role in consuming part of the nutrient input of the tree or even supplementing the tree's budget in the form of detritus, i.e. whether the interaction is parasitic, commensalistic or mutualistic. Here we elucidated the processes through which *Tillandsia* acquires nitrogen,

determine the role that Spanish moss decomposition plays in contributing N to the soil, and resolve the nature of the symbiosis between Spanish moss and southern live oaks.

We found that Spanish moss did not consume nutrients from the tree as a result of leaching, nor was it specifically dependent on dust and excrements or microbial nitrogen fixation as a nutrient source. Spanish moss' nitrogen intake was mostly dependent on atmospheric deposition. We could also conclude that Spanish moss was not intercepting nitrogen leaking from the tree. Strikingly, we found that Spanish moss contributed up to 26% of the tree's above-ground nitrogen input to the top soil layer, which constitutes an ecologically significant portion in this nitrogen limited system. The interaction between Spanish moss and the host tree can therefore be considered a mutualism, as Spanish moss non-trophically benefits from the structure of the host for its habitat, and the host tree benefits from Spanish moss as an additional slow release fertilizer in the form of detritus (Table 1, Chapter 1). This study highlights the evolutionary strength of species to move away from negative interactions such as competition and parasitism towards neutral and positive interactions such as niche partitioning and, under nutrient stress, mutualism. Other foundation species, theoretically in particular in nutrient-poor systems, might therefore also be dependent on their facilitated species for their nutrient input (Derksen-Hooijberg *et al.* 2018).

Dispersal breakdown as a result of hunting

What can be a beneficial interaction when studying interspecific interaction at a small scale, may well result in a different type of interaction on the large scale or in the longer term (Chapter 3). It is therefore important to study also at a larger spatial or temporal scale whether a relationship is shaped mostly by trophic or non-trophic interactions. In Chapter 5, the majority of the mammal seed disperser community was removed as a result of hunting, while being protected in a nearby area, which can be viewed as a large-scale exclosure study. We elucidated the effects of the consequent dispersal breakdown and the density dependent responses of host-specific predators by quantifying the population dynamics using the complete life cycle of a palm species *Attalea butyraceae* dispersed by mammals in hunted and non-hunted sites. To accomplish this, we explored how local plant species abundance and population dynamics are jointly influenced by seed dispersal, seed density and seedling mortality using a generic theoretical model. We used this model to elucidate the contribution of both the non-trophic effect of dispersal and the trophic effect of seed predation on the dynamics of the interaction between the palm, its dispersers and host-specific enemies.

Attalea butyraceae's range stretches out across the entire Central American region and the species is defined as a hyperabundant species in the neotropics (ter Steege *et al.* 2013). As its complex crown structure provides a large number of microhabitats to invertebrates and

vertebrates (Abad-Franch *et al.* 2005; Kanegae *et al.* 2012; Poveda *et al.* 2017) the palm can be seen as an important tropical foundation species. Because it is bearing fruit throughout the year, it provides a large portion of the diet of mammals in neotropical forests (see Desteven *et al.* 1987; Jansen *et al.* 2014). In return, *Attalea* itself is dependent upon these frugivores for its dispersal (Wright & Duber 2001; Jansen *et al.* 2012; Jansen *et al.* 2014). The scatter-hoarding rodents' caching and re-caching behavior disperses the large seeds of *Attlaea* the farthest, while it is at the same time the main seed predator (Silvius 2002; Visser *et al.* 2011).

The removal of seed dispersers by hunting influenced the population dynamics of the *Attalea* species in a number of ways. First of all, we demonstrate hunting dramatically reduces dispersal distances. Through this lack of dispersal, seed and seedling densities increased more than 7 times. Monospecific seedling mats were no exception in the hunted forest (personal observation). Host-specific predators and pathogens did not compensate for the increased seed densities, increasing local palm abundances to five times the control abundances. Lack of compensation by predators and pathogens goes against the consensus amongst many tropical ecologists that co-existence in species rich forests can be largely explained by overcompensating negative density dependent processes (Janzen 1970; Freckleton & Lewis 2006; Bagchi *et al.* 2010). Locally these increased abundances of one species could lead to a loss in biodiversity, as the increased abundance of *Attalea* leads to reduced habitat space for other species (Wright & Duber 2001). Apart from the effect of increased *Attalea* numbers on the local scale, it is also important to note that the lack of dispersal will have negative effects on *Attalea* fitness on the large scale, as it will not be able to disperse sufficiently. The aggregation of relative plants may also depress the genetic landscape, as genetic exchange will decrease.

Perspectives and future research

The integration of trophic and non-trophic interactions in multilayered models, although necessary for a better understanding of ecosystem functioning and dynamics, remains a daunting task. One of the issues that scientists need to tackle is the lack of sufficient insight into the strength and relative contributions of separate key trophic and non-trophic interactions.

In this thesis, I have taken a first step to disentangle the many trophic and non-trophic interactions within communities of organisms. Gathering sufficient data to conclusively model all trophic and non-trophic interactions in ecosystems is currently very challenging, and sometimes virtually impossible due to the complexity of the interactions within communities and ecosystems. Therefore, it may be helpful to acknowledge the complexity of these systems and only focus on finding broad-scale patterning and emergent properties, like I did in Chapter

2. These types of studies may point to key interactions and mechanisms that underlie the dynamics of the systems and allow for simplified models to be constructed. I have shown here that trophic and non-trophic facilitation by foundation species induces pervasive changes on a food web level. Finding such emergent patterns will allow us to start predicting the limitations and the dynamics of the systems, even when we cannot model all processes underlying these patterns. In addition, trying to understand these patterns in a theoretical framework (Chapter 5) will automatically lead to new hypotheses that can be tested empirically.

In this thesis I specifically focused on disentangling trophic versus non-trophic interactions in foundation species-dominated systems. The facilitating effect of foundation species is often contributed to their role in stress mitigation, habitat provisioning and accumulating external food sources (e.g. captured organic matter like POM and epiphyton). On the basis of my research I suggest that a fourth pathway, the trophic facilitation by the foundation species' tissue as a food source in the form of detritus, is often disregarded or underestimated. The facilitative effect of Spanish moss is, next to habitat provisioning, for an important part due to the trophic effect of decomposing plant tissue (Chapter 3). Also *Attalea* palms are specifically facilitating mammals trophically while providing habitat for the invertebrate community (Abad-Franch *et al.* 2005; Jansen *et al.* 2014). To further our understanding of the facilitating effects of foundation species, future research should take into account the origin of detritus, whether it is internally produced by the foundation species itself or externally (Moore *et al.* 2004; Miller & Page 2012). As I show in Chapter 3, overlooking or disregarding this effect may strongly underestimate the role of trophic or non-trophic food provisioning, which is key to progress towards an integration of the two. In addition, we need to study to what extent the live tissue of the foundation species is consumed, to integrate brown and green food webs and non-trophic interactions.

Lastly, I would like to highlight the importance of investigating trophic and non-trophic interactions on different spatial or temporal scales. Effects on a small spatial or temporal scale may seem insignificant. However, when integrated into the whole it may strongly influence community and ecosystem dynamics. When looking on short temporal scales, Spanish moss does not seem to influence its host. On larger temporal scales, however, we showed that via detritus Spanish moss had a significant influence on its host (Chapter 4). Similarly, it is important to take the whole life cycle of a species into account when judging the effects of interactions only for a single life stage (Chapter 5). Positive interactions in earlier life stages may be (partly) offset in a later life stage as we described in our study of *Attalea*. Long term datasets allow us to empirically study large scale patterns that allows us to define hypothetical key-processes that can then be studied in detailed experimental settings. For example, large scale studies (e.g. Knapp & Smith 2001; Roscher *et al.* 2004; Hallmann *et al.* 2017) have provided many important insights we would not be able to find without these specific efforts.

Conclusions

I here show that non-trophic interactions play an important role in foundation species-dominated systems. Specifically, I found that habitat modification mitigated stress and provided vital habitat structures for associated species in a wide range of ecosystems, strongly enhancing biodiversity. Secondly, I demonstrate that the trophic role of foundation species may be much larger than is often assumed. This is because the detritus of these organisms can serve as an important food source for detritivores that in turn can be a food source for higher trophic levels. Thirdly, I show that foundation species are also reciprocally influenced by species they facilitate. In my studies interactions were shown to be mutualistic in the form of nutrients or dispersal by host species. It remains a challenge to study all underlying processes in a system, as the interaction with one interaction partner may be changed by the effect of another interaction partner, as is highlighted in Chapter 5 where the bruchid beetles' seed predation behavior partly compensated the loss of seed predation by rodents.

In conclusion, I have taken important steps in assessing the contributions of trophic and non-trophic interactions in different types of interspecific relations. Zooming in to study specific key interactions, and zooming out again on species interactions to look for emergent patterns, like I have done in this thesis, leads to a better understanding of ecosystem functioning. This combined approach is not only needed to disentangle complex species interactions that form communities and ecosystems, but also to signal important process changes caused by anthropogenic stressors such as climate change and species extinction due to land use change.

7. References

Vervang door opgemaakte versie

A

Abad-Franch, F., F.S. Palomeque, H.M. Aguilar & M.A. Miles (2005). Field ecology of sylvatic *Rhodnius* populations (Heteroptera, Triatominae): risk factors for palm tree infestation in western Ecuador. *Tropical Medicine & International Health*, 10, 1258-1266.

Abril, A. & E. Bucher (2009). A comparison of nutrient sources of the epiphyte *Tillandsia capillaris* attached to trees and cables in Cordoba, Argentina. *Journal of arid environments*, 73, 393-395.

Aerts, R. (1996). Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*, 597-608.

Ali, A., and E. R. Yan (2017). Relationships between biodiversity and carbon stocks in forest ecosystems: a systematic literature review. *Tropical Ecology*, 58, 1-14.

Allesina, S., D. Alonso & M. Pascual (2008) A general model for food web structure. *Science*, 320**,** 658-661.

Altieri, A. H., B. R. Silliman & M. D. Bertness (2007) Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *American Naturalist*, 169**,** 195-206.

Anderson, D.R. & K.P. Burnham (2002). Avoiding pitfalls when using information-theoretic methods. *Journal of Wildlife Management*, 66, 912-918.

Angelini, C. & B.R. Silliman (2014) Secondary foundation species as drivers of trophic and functional diversity: evidence from a tree-epiphyte system. *Ecology*, 95**,** 185-196.

Angelini, C. & K.L. Briggs (2015). Spillover of secondary foundation species transforms community structure and accelerates decomposition in oak savannas. *Ecosystems*, 18, 780-791.

Angelini, C., A.H. Altieri, B.R. Silliman & M.D. Bertness (2011) Interactions among foundation species and their consequences for community organization, biodiversity, and conservation. *Bioscience*, 61**,** 782-789.

Angelini, C., J.N. Griffin, J. van de Koppel, L.P.M. Lamers, A.J.P. Smolders, M. Derksen-Hooijberg *et al.* (2016). A keystone mutualism underpins resilience of a coastal ecosystem to drought. *Nature Communications*, 7, 12473.

Angelini, C., T. van der Heide, J.N. Griffin, J.P. Morton, M. Derksen-Hooijberg, L.P.M. Lamers, *et al.* (2015). Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. *Proceedings. Biological sciences / The Royal Society*, 282, 20150421.

Arroyo-Rodriguez, V., E. Pineda, F. Escobar & J. Benitez-Malvido (2009). Value of small patches in the conservation of plant-species diversity in highly fragmented rainforest. *Conservation Biology*, 23, 729-739.

Avin, C., M. Borokhovich, B. Haeupler & Z. Lotker (2015) Self-adjusting grid networks to minimize expected path length. *Theoretical Computer Science,* 584**,** 91-102.

B

Bagchi, R., P.A. Henrys, P.E Brown, D. Burslem, P.J. Diggle, C.V.S. Gunatilleke, *et al.* (2011). Spatial patterns reveal negative density dependence and habitat associations in tropical trees. *Ecology*, 92, 1723-1729.

Bagchi, R., T. Swinfield, R.E. Gallery, O.T. Lewis, S. Gripenberg, L. Narayan, *et al.* (2010). Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. *Ecology Letters*, 13, 1262-1269.

Baiser, B., N. Whitaker & A.M. Ellison (2013) Modeling foundation species in food webs. *Ecosphere,* 4**,** 1-14.

Barabas, G., M.J. Michalska-Smith & S. Allesina (2017). Self-regulation and the stability of large ecological networks. *Nature Ecology & Evolution*, 1, 1870-1875.

Beckman, N.G. & H.C. Muller-Landau (2007). Differential effects of hunting on pre-dispersal seed predation and primary and secondary seed removal of two neotropical tree species. *Biotropica,* 39, 328-339.

Beckman, N.G., C. Neuhauser & H.C. Muller-Landau (2012). The interacting effects of clumped seed dispersal and distance- and density-dependent mortality on seedling recruitment patterns. *Journal of Ecology*, 100, 862-873.

Begon, M., J.L. Harper & C.R. Townsend (1996). *Ecology. Individuals, Populations and Communities*. Blackwell, Cambridge.

Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller & F. Courchamp (2012) Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15**,** 365-377.

Bello, C., M. Galetti, M.A. Pizo, L.F.S. Magnago, M.F. Rocha, R.A.F. Lima, *et al.* (2015). Defaunation affects carbon storage in tropical forests. *Science Advances*, 1, e1501105.

Benzing, D. (1981). The population dynamics of *Tillandsia circinnata* (Bromeliaceae): cypress crown colonies in southern Florida. *Selbyana,* 5, 256-263.

Benzing, D.H. & J. Seemann (1978). Nutritional piracy and host decline: a new perspective on the epiphyte-host relationship. *Selbyana,* 2, 133-148.

Benzing, D.H. (1990). Vascular epiphytes: general biology and related biota. Cambridge University Press.

Berlow, E. L., A.M. Neutel, J.E. Cohen, P.C. de Ruiter, B. Ebenman, M. Emmerson, *et al.* (2004) Interaction strengths in food webs: issues and opportunities. *Journal of Animal Ecology,* 73**,** 585-598.

Berlow, E.L., J.A. Dunne, N.D. Martinez, P.B. Stark, R.J. Williams & U. Brose (2009). Simple prediction of interaction strengths in complex food webs. *Proceedings of the National Academy of Sciences of the United States of America,* 106, 187-191.

Bertness, M.D. & R. Callaway (1994) Positive interactions in communities. *Trends in Ecology & Evolution,* 9**,** 191-193.

Bertness, M.D., G.H. Leonard, J.M. Levine, P.R. Schmidt & A.O. Ingraham (1999) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. *Ecology,* 80**,** 2711-2726.

Blick, R. & K. Burns (2009). Network properties of arboreal plants: are epiphytes, mistletoes and lianas structured similarly? *Perspectives in Plant Ecology, Evolution and Systematics*, 11, 41-52.

Bolker, B. (2008). *Ecological Models and Data in R.* Princeton University Press, Princeton, NJ.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, *et al.* (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127-135.

Bologna, P.A.X. & K.L. Heck (1999) Macrofaunal associations with seagrass epiphytes - Relative importance of trophic and structural characteristics. *Journal of Experimental Marine Biology and Ecology*, 242**,** 21-39.

Bowman, W.D., S.D. Hacker & M.L. Cain (2017). *Ecology*. Fourth edn. Sinauer Associates, Sutherland, USA.

Brighigna, L., P. Montaini, F. Favilli & A.C. Trejo (1992). Role of the nitrogen-fixing bacterial microflora in the epiphytism of *Tillandsia* (Bromeliaceae). *American Journal of Botany*, 79, 723-727.

Brodie, J.F., O.E. Helmy, W.Y. Brockelman & J.L Maron (2009). Bushmeat poaching reduces the seed dispersal and population growth rate of a mammal-dispersed tree. *Ecological Applications*, 19, 854-863.

Bronstein, J.L. (2009). The evolution of facilitation and mutualism. *Journal of Ecology*, 97, 1160-1170.

Brose, U., T. Jonsson, E.L. Berlow, P. Warren, C. Banasek-Richter, L.F. Bersier *et al.* (2006). Consumer-resource body-size relationships in natural food webs. *Ecology*, 87, 2411-2417.

Bruno, J.F., J.J. Stachowicz & M.D. Bertness (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, 18**,** 119-125.

Bruno, J.F. & M.D. Bertness (2001). Habitat modification and facilitation in benthic marine communities. In: *Marine community ecology* (eds. M.D., B, Hay, ME & Gaine, SD). Sinauer Suntherland, USA, 201-218.

Buckland, S.T., K.P. Burnham & N.H. Augustin (1997). Model selection: An integral part of inference. *Biometrics*, 53, 603-618.

Bulleri, F., J.F. Bruno, B.R. Silliman & J.J. Stachowicz (2016) Facilitation and the niche: implications for coexistence, range shifts and ecosystem functioning. *Functional Ecology*, 30**,** 70-78.

Bustamante, M., E. Medina, G. Asner, G. Nardoto & D. Garcia-Montiel (2006). Nitrogen cycling in tropical and temperate savannas. In: *Nitrogen cycling in the Americas: natural and anthropogenic influences and controls*. Springer, 209-237.

C

Callaway, R.M., K.O. Reinhart, G.W. Moore, D.J. Moore & S.C. Pennings (2002). Epiphyte host preferences and host traits: mechanisms for species-specific interactions. *Oecologia*, 132, 221-230.

Campanya-Llovet, N., P.V.R. Snelgrove, and C.C. Parrish. (2017). Rethinking the importance of food quality in marine benthic food webs. *Progress in Oceanography*, 156, 240-251.

Caswell, H. (2001). Matrix population models: Construction, analysis and interpretation. Sinauer Sunderland, USA.

Caughlin, T.T., J.M. Ferguson, J.W. Lichstein, P.A. Zuidema, S. Bunyavejchewin & D.J. Levey (2015). Loss of animal seed dispersal increases extinction risk in a tropical tree species due to pervasive negative density dependence across life stages. *Proceedings of the Royal Society B-Biological Sciences*, 282, 20142095.

Chazdon, R.L. (2003) Tropical forest recovery: Legacies of human impact and natural disturbances. *Perspectives in Plant Ecology Evolution and Systematics*, 6**,** 51-71.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31, 343-366.

Christianen, M.J.A., T. van der Heide, S J. Holthuijsen, K.J. van der Reijden, A.C.W. Borst & H. Olff (2017) Biodiversity and food web indicators of community recovery in intertidal shellfish reefs. *Biological Conservation*, 213**,** 317-324.

Clark, D.A. & D.B. Clark (1992). Life-history diversity of canopy and emergent trees in a neotropical rain-forest. *Ecological Monographs*, 62, 315-344.

Clark, D.B., D.A. Clark & P.M. Rich (1993). Comparative-analysis of microhabitat utilization by saplings of 9 tree species in neotropical rain-forest. *Biotropica*, 25, 397-407.

Clark, J.S., M. Silman, R. Kern, E. Macklin & J. HilleRisLambers (1999). Seed dispersal near and far: Patterns across temperate and tropical forests. *Ecology*, 80, 1475-1494.

Coker, D.J., S.K. Wilson & M.S. Pratchett (2014). Importance of live coral habitat for reef fishes. *Reviews in Fish Biology and Fisheries*, 24**,** 89-126.

Comita, L.S., H.C. Muller-Landau, S. Aguilar & S.P. Hubbell (2010). Asymmetric density dependence shapes species abundances in a tropical tree community. *Science*, 329, 330-332.

Comita, L.S., S.A. Queenborough, S.J. Murphy, J.L. Eck, K.Y. Xu, M. Krishnadas *et al.* (2014). Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology*, 102, 845-856.

Cook, B.I., E.R. Cook, J.E. Smerdon, R. Seager, A.P. Williams, S. Coats *et al.* (2016). North American megadroughts in the Common Era: reconstructions and simulations. *Wiley Interdisciplinary Reviews-Climate Change*, 7, 411-432.

Corlett, R.T. (2007). The impact of hunting on the mammalian fauna of tropical Asian forests. *Biotropica*, 39, 292-303.

Cornelissen, J.H.C., S.I. Lang, N.A. Soudzilovskaia & H.J. During (2007). Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*, 99, 987-1001.

Coxson, D. & N. Nadkarni (1995). Ecological roles of epiphytes in nutrient cycles of forest ecosystems. In: *Forest canopies. Academic Press, San Diego*, 495-543.

Culot, L., C. Bello, J.L.F. Batista, H.T.Z. do Couto & M. Galetti (2017). Synergistic effects of seed disperser and predator loss on recruitment success and long-term consequences for carbon stocks in tropical rainforests. *Scientific Reports*, 7, 7662.

D

Darwin, C. (1859). *On the origin of species*. John Murray, London.

Davies, S.J., P.A. Palmiotto, P.S. Ashton, H.S. Lee & J.V. Lafrankie (1998). Comparative ecology of 11 sympatric species of Macaranga in Borneo: tree distribution in relation to horizontal and vertical resource heterogeneity. *Journal of Ecology*, 86, 662-673.

Dawkins, H.C. & D.R.B. Field (1978). A long-term surveillance system for British woodland vegetation. *Occasional Papers of the Commonwealth Forestry Institute*, 1.

Dayton, P.K. (1972). Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In: *Proceedings of the Colloquium on Conservation Problems in Antarctica* (ed. Parker, B). Allen Press Lawrence, Kansas.

De Graaf, M.C., R. Bobbink, N.A. Smits, R. Van Diggelen & J.G. Roelofs (2009). Biodiversity, vegetation gradients and key biogeochemical processes in the heathland landscape. *Biological conservation*, 142, 2191-2201.

de Kroon, H., J. van Groenendael & J. Ehrlen (2000). Elasticities: A review of methods and model limitations. *Ecology*, 81, 607-618.

DeAngelis, D.L. (1975). Stability and connectance in food web models. *Ecology*, 56, 238-243.

DeLuca, T.H., O. Zackrisson, M.C. Nilsson & A. Sellstedt (2002). Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature*, 419, 917-920.

Derksen-Hooijberg, M., C. Angelini, L.P.M. Lamers, A.C.W. Borst, A. Smolders, J.R.H Hoogveld *et al.* (2018). Mutualistic interactions amplify saltmarsh restoration success. *Journal of Applied Ecology*, 55, 405-414.

Desteven, D., D.M Windsor, F.E. Putz & B. Deleon (1987). Vegetative and reproductive phenologies of a palm assemblage in Panama. *Biotropica*, 19, 342-356.

Dibble, E. D., K. J. Killgore & G. O. Dick (1996). Measurement of plant architecture in seven aquatic plants. *Journal of Freshwater Ecology*, 11, 311-331.

Dicke, M. (1994). Local and systemic production of volatile herbivore-induced terpenoids - their role in plant-carnivore mutualism. *Journal of Plant Physiology*, 143, 465-472.

Dillon, K.S. & J.P. Chanton (2005). Nutrient transformations between rainfall and stormwater runoff in an urbanized coastal environment: Sarasota Bay, Florida. *Limnology and Oceanography*, 50, 62-69.

Dirzo, R., E. Mendoza & P. Ortíz (2007). Size-related differential seed predation patterns in a heavily defaunated neotropical rain forest. *Biotropica*, 39, 355–362.

Dirzo, R., H.S. Young, M. Galetti, G. Ceballos, N.J.B. Isaac & B. Collen (2014). Defaunation in the Anthropocene. *Science*, 345, 401-406.

Donadi, S., T. van der Heide, E.M. van der Zee, J.S. Eklof, J. van de Koppel, E.J. Weerman, *et al.* (2013) Cross-habitat interactions among bivalve species control community structure on intertidal flats. *Ecology*, 94**,** 489-498.

Donatti, C.I., P.R. Guimaraes & M. Galetti (2009). Seed dispersal and predation in the endemic Atlantic rainforest palm *Astrocaryum aculeatissimum* across a gradient of seed disperser abundance. *Ecological Research*, 24, 1187-1195.

Donker, J. J. A., M. van der Vegt & P. Hoekstra (2013) Wave forcing over an intertidal mussel bed. *Journal of Sea Research*, 82**,** 54-66.

Duncan, W.H. & M.B. Duncan (1988). *Trees of the southeastern United States*. University of Georgia Press.

Dunne, J.A., R.J. Williams & N.D. Martinez (2002a). Food-web structure and network theory: The role of connectance and size. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 12917-12922.

Dunne, J.A., R.J. Williams & N.D. Martinez (2002b). Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters*, 5, 558-567.

Dunne, J.A. & R.J. Williams (2009). Cascading extinctions and community collapse in model food webs. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 364, 1711-1723.

E

Effiom, E.O., G. Nunez-Iturri, H.G. Smith, U. Ottosson & O. Olsson (2013). Bushmeat hunting changes regeneration of African rainforests. *Proceedings of the Royal Society B-Biological Sciences*, 280, 20130246.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, *et al.* (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, 3**,** 479-486.

Elton, C.S. (1927). *Animal Ecology*. the Macmillan Company, New York.

F

Felix, J.D., E.M. Elliott & D.A. Gay (2017). Spatial and temporal patterns of nitrogen isotopic composition of ammonia at US ammonia monitoring network sites. *Atmospheric Environment*, 150, 434-442.

Felix, J.D., E.M. Elliott, T. Gish, R. Maghirang, L. Cambal & J. Clougherty (2014). Examining the transport of ammonia emissions across landscapes using nitrogen isotope ratios. *Atmospheric environment*, 95, 563-570.

Ferreiro, N., C. Feijoo, A. Giorgi & L. Leggieri (2011). Effects of macrophyte heterogeneity and food availability on structural parameters of the macroinvertebrate community in a Pampean stream. *Hydrobiologia*, 664, 199-211.

Filazzola, A., M. Westphal, M. Powers, A.R. Liczner, D.A. Woollett, B. Johnson & C.J. Lortie (2017) Non-trophic interactions in deserts: Facilitation, interference, and an endangered lizard species. *Basic and Applied Ecology*, 20**,** 51-61.

Finzi, A.C. (2009). Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry*, 92, 217-229.

Flores-Palacios, A. (2016). Does structural parasitism by epiphytes exist? A case study between *Tillandsia recurvata* and *Parkinsonia praecox*. *Plant Biology*, 18, 463-470.

Foster, R.B. & N.V.L. Brokaw (1982). Structure and history of the vegetation of Barro Colorado Island. In: *The Ecology of a Tropical Forest: Seasonal Rhythems and Long Term Changes*. Smithsonian Institutional Press Washington D.C., 67–81.

Francisco, T.M., M.L. Garbin, C. De Toledo Castanho & C.R. Ruiz-Miranda (2018). An overview on epiphytism as a direct mechanism of facilitation in tropical forests. *Tropical Ecology*, 59, 1-9.

Freckleton, R.P. & O.T. Lewis (2006). Pathogens, density dependence and the coexistence of tropical trees. *Proceedings of the Royal Society B-Biological Sciences*, 273, 2909-2916.

G

Garth, R.E. (1964). The ecology of Spanish moss (*Tillandsia usneoides*): its growth and distribution. *Ecology*, 45, 470-481.

Gartner, A., F. Tuya, P.S. Lavery & K. McMahon (2013). Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. *Journal of Experimental Marine Biology and Ecology*, 439, 143-151.

Gause, G.F. (1932). Experimental studies on the struggle for existence. *Journal of Experimental Biology*, 9, 389-402.

Gedan, K.B., B.R. Silliman & M.D. Bertness (2009) Centuries of human-driven change in salt marsh ecosystems. *Annual Review of Marine Science*, 1**,** 117-141.

Gilad, E., J. von Hardenberg, A. Provenzale, M. Shachak & E. Meron (2004) Ecosystem engineers: From pattern formation to habitat creation. *Physical Review Letters*, 93, 098105.

Gilad, E., J. von Hardenberg, A. Provenzale, M. Shachak & E. Meron (2007) A mathematical model of plants as ecosystem engineers. *Journal of Theoretical Biology*, 244**,** 680-691.

Gilbert, A.J. (2009). Connectance indicates the robustness of food webs when subjected to species loss. *Ecological Indicators*, 9, 72-80.

Govenar, B. (2010). Shaping vent and seep communities: Habitat provision and modification by foundation species. In: *The Vent and Seep Biota* (ed. Kiel, S). Springer Netherlands, pp. 403-432.

Granados, A., J.F. Brodie, H. Bernard & M.J. O'Brien (2017). Defaunation and habitat disturbance interact synergistically to alter seedling recruitment. *Ecological Applications*, 27, 2092-2101.

Grant, P.R. & B.R. Grant (2006). Evolution of character displacement in Darwin's finches. *Science*, 313, 224-226.

Grasshoff, K. & H. Johannsen (1972). A new sensitive and direct method for the automatic determination of ammonia in sea water. *Journal du Conseil*, 34, 516-521.

Grueber, C.E., S. Nakagawa, R.J. Laws & I.G. Jamieson (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, 24, 699-711.

Grutters, B.M.C., B.J.A. Pollux, W. Verberk & E.S. Bakker (2015) Native and non-native plants provide similar refuge to invertebrate prey, but less than artificial plants. *Plos One*, 10, e0124455.

Güsewell, S. (2004). N:P ratios in terrestrial plants: variation and functional significance. *New phytologist*, 164, 243-266.

Gutierrez, J.L., C.G. Jones, D.L. Strayer & O.O. Iribarne (2003) Mollusks as ecosystem engineers: The role of shell production in aquatic habitats. *Oikos*, 101**,** 79-90.

H

Hallmann, C.A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan *et al.* (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *Plos One*, 12, e0185809.

Halpern, B.S., B.R. Silliman, J.D. Olden, J.P. Bruno & M.D. Bertness (2007). Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment*, 5, 153-160.

Hamrick, J.L. (2004). Response of forest trees to global environmental changes. *Forest Ecology and Management*, 197, 323-335.

Harrison, R.D. (2011). Emptying the forest: Hunting and the extirpation of wildlife from tropical nature reserves. *Bioscience*, 61, 919-924.

Harrison, R.D., S. Tan, J.B. Plotkin, F. Slik, M. Detto, T. Brenes *et al.* (2013). Consequences of defaunation for a tropical tree community. *Ecology Letters*, 16, 687-694.

Hastings, A., J.E. Byers, J.A. Crooks, K. Cuddington, C.G. Jones, J.G. Lambrinos *et al.* (2007). Ecosystem engineering in space and time. *Ecology Letters*, 10, 153-164.

Hietz, P. & W. Wanek (2003). Size-dependent variation of carbon and nitrogen isotope abundances in epiphytic bromeliads. *Plant Biology*, 5, 137-142.

Holland, J.N. & D.L. DeAngelis (2009). Consumer-resource theory predicts dynamic transitions between outcomes of interspecific interactions. *Ecology Letters*, 12, 1357-1366.

Holland, J.N. & D.L DeAngelis (2010). A consumer-resource approach to the density-dependent population dynamics of mutualism. *Ecology*, 91, 1286-1295.

Holling, C.S. (1959). The components of predation as revealed by a study of small- mammal predation of the European pine sawfly. *The Canadian entomologist*, 91, 234-261.

Holling, C.S. (1961). Principles of insect predation. *Annual Review of Entomology*, 6, 163-182

Howe, H.F. & J. Smallwood (1982). Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, 13, 201-228.

Hutchinson, G.E. (1961). The Paradox of the Plankton. *American Naturalist*, 95, 137-145.

J

Jacquet, C., C. Moritz, L. Morissette, P. Legagneux, F. Massol, P. Archambault *et al.* (2016). No complexity-stability relationship in empirical ecosystems. *Nature Communications*, 7, 12573.

Jansen, P.A., F. Bongers & L. Hemerik (2004). Seed mass and mast seeding enhance dispersal by a neotropical scatter-hoarding rodent. *Ecological Monographs,* 74, 569-589.

Jansen, P.A., B.T. Hirsch, W.J. Emsens, V. Zamora-Gutierrez, M. Wikelski & R. Kays (2012). Thieving rodents as substitute dispersers of megafaunal seeds. *Proceedings of the National Academy of Sciences of the United States of America,* 109, 12610-12615.

Jansen, P.A., M.D. Visser, S.J. Wright, G. Rutten & H.C. Muller-Landau (2014). Negative density dependence of seed dispersal and seedling recruitment in a Neotropical palm. *Ecology Letters*, 17, 1111-1120.

Janzen, D.H. (1970). Herbivores and the number of tree species in tropical forests. *American Naturalist,* 104, 501-528.

Janzen, D.H. (1974). Deflowering of central-America. *Natural History*, 83, 48-53.

Janzen, D.H. (1980). Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *Journal of Ecology,* 68, 929-952.

Jaschinski, S., D.C. Brepohl & U. Sommer (2011) The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus L.*): stable isotope and fatty acid analyses. *Aquatic Sciences,* 73**,** 91-101.

Jeppesen, E., M. Sondergaard, M. Sondergaard & K. Christofferson (1992). *The structuring role of submerged macrophytes in lakes*. New York: Springer.

Johansson, D. (1974). Ecology of vascular epiphytes in West African rain forest. In: *Acta Phytogeographica Suecica*. Uppsala University Uppsala, Sweden.

Jones, C. G., J.L. Gutierrez, J.E. Byers, J.A. Crooks, J.G. Lambrinos & T.S. Talley (2010) A framework for understanding physical ecosystem engineering by organisms. *Oikos,* 119**,** 1862-1869.

Jones, C.G., J.H. Lawton & M. Shachak (1994). Organisms as ecosystem engineers. *Oikos*, 69, 373-386.

Jongejan, F. & G. Uilenberg (2004). The global importance of ticks. *Parasitology*, 129, S3-S14.

Jorgensen, S.E., B.C. Patten & M. Straskraba (1992). Ecosystems emerging - Toward an ecology of complex-systems in a complex future. *Ecological Modelling*, 62, 1-27.

K

Kaehler, S., E.A. Pakhomov &C.D. McQuaid (2000). Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by delta C-13 and delta N-15 analysis. *Marine Ecology Progress Series*, 208, 13-20.

Kaehler, S., E.A. Pakhomov, R.M. Kalin & S. Davis (2006). Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. *Marine Ecology Progress Series*, 316, 17-22.

Kaiser, W., E. Huguet, J. Casas, C. Commin & D. Giron (2010). Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society B-Biological Sciences*, 277, 2311-2319.

Kamphake, L.J., S.A. Hannah & J.M. Cohen (1967). Automated analysis for nitrate by hydrazine reduction. *Water research*, 1, 205-216.

Kanegae, M.F., G. Levy & S.R. Freitas (2012). Habitat use by Sharp-tailed Tyrant (*Culicivora caudacuta*), and Cock-tailed Tyrant (*Alectrurus tricolor*) in the Cerrado of Southeastern Brazil. *Revista Brasileira De Ornitologia*, 20, 52-58.

Kefi, S., E.L. Berlow, E.A. Wieters, L.N. Joppa, S.A. Wood, U. Brose *et al.* (2015). Network structure beyond food webs: mapping non-trophic and trophic interactions on Chilean rocky shores. *Ecology*, 96, 291-303.

Kefi, S., E.L. Berlow, E.A. Wieters, S.A. Navarrete, O.L. Petchey, S.A. Wood *et al.* (2012). More than a meal ... integrating non-feeding interactions into food webs. *Ecology Letters*, 15, 291-300.

Kiers, E.T., T.M. Palmer, A.R Ives, J.F. Bruno & J.L. Bronstein (2010). Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters*, 13, 1459-1474.

Killingbeck, K.T. (1996). Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology*, 77, 1716-1727.

Klecka, J. & D.S. Boukal (2014) The effect of habitat structure on prey mortality depends on predator and prey microhabitat use. *Oecologia*, 176**,** 183-191.

Klinger, R. & M. Rejmanek (2009). The numerical and functional responses of a granivorous rodent and the fate of Neotropical tree seeds. *Ecology*, 90, 1549-1563.

Knapp, A.K. & M.D. Smith (2001). Variation among biomes in temporal dynamics of aboveground primary production. *Science*, 291, 481-484.

Koopmans, C.J., D. vanDam, A. Tietema & J.M. Verstraten (1997). Natural N-15 abundance in two nitrogen saturated forest ecosystems. *Oecologia*, 111, 470-480.

Kovalenko, K.E., S.M. Thomaz & D.M. Warfe (2012) Habitat complexity: approaches and future directions. *Hydrobiologia*, 685**,** 1-17.

Kuiper, J.J., C. van Altena, P.C. de Ruiter, L.P.A. van Gerven, J.H. Janse & W.M. Mooij (2015) Food-web stability signals critical transitions in temperate shallow lakes. *Nature Communications,* 6, 7727.

Kurten, E.L., S.J. Wright & W.P. Carson (2015). Hunting alters seedling functional trait composition in a Neotropical forest. *Ecology*, 96, 1923-1932.

Kuznetsova, A., P. Bruun Brockhoff & R. Haubo Bojesen Christensen (2015). *lmerTest: Tests in Linear Mixed Effects Models.*, R package version 2.0-29.

L

Lang, G.E., W.A. Reiners & R.K. Heier (1976). Potential alteration of precipitation chemistry by epiphytic lichens. *Oecologia*, 25, 229-241.

Li, Y. H., U. Brose, K. Meyer, and B.C. Rall (2017). How patch size and refuge availability change interaction strength and population dynamics: a combined individual- and population-based modeling experiment. *Peerj*,5, e2993.

Lindeman, R.L. (1942). The trophic-dynamic aspect of ecology. *Ecology*, 23, 399-417.

Lindo, Z., M.C. Nilsson & M.J. Gundale (2013). Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. *Global Change Biology*, 19, 2022-2035.

Lindow, S.E. & M.T. Brandl (2003). Microbiology of the phyllosphere. *Applied and environmental microbiology*, 69, 1875-1883.

Liu, X.Y., K. Koba, A. Makabe, X.D. Li, M. Yoh & C.Q. Liu (2013). Ammonium first: natural mosses prefer atmospheric ammonium but vary utilization of dissolved organic nitrogen depending on habitat and nitrogen deposition. *New Phytologist*, 199, 407-419.

Lodge, D.M. (1991) Herbivory on freshwater macrophytes. *Aquatic Botany,* 41**,** 195-224.

Loke, L.H.L. & P.A. Todd (2016). Structural complexity and component type increase intertidal biodiversity independently of area. *Ecology*, 97, 383-393.

Lotka, A.J. (1910). Contribution to the Theory of Periodic Reaction. *J. Phys. Chem*., 14, 271–274.

M

Macarthur, R.H. (1958). Population ecology of some warblers of northeastern coniferous forests. *Ecology*, 39, 599-619.

Markl, J.S., M. Schleuning, P.M. Forget, P. Jordano, J.E. Lambert, A. Traveset *et al.* (2012). Meta-analysis of the effects of human disturbance on seed dispersal by animals. *Conservation Biology*, 26, 1072-1081.

Matias, M.G., A.J. Underwood, D.F. Hochuli & R.A. Coleman (2010). Independent effects of patch size and structural complexity on diversity of benthic macroinvertebrates. *Ecology*, 91, 1908-1915.

MEA (2005). Millennium Assessment - Ecoystems and Human Well-being. ed. I. Press. Washington D.C.

Melo, M.M., C.M. Silva, C S. Barbosa, M.C. Morais, P.E.R. D'Anunciacao, V.X. da Silva, *et al.* (2016). Fragment edge and isolation affect the food web: effects on the strength of interactions among trophic guilds. *Biota Neotropic*, 16, e20150088.

Miller, R.J., H.M. Page & D.C. Reed (2015) Trophic versus structural effects of a marine foundation species, giant kelp (*Macrocystis pyrifera*). *Oecologia*, 179**,** 1199-1209.

Miller, R.J. & H.M. Page (2012). Kelp as a trophic resource for marine suspension feeders: A review of isotope-based evidence. *Marine Biology*, 159, 1391-1402.

Milne, B.T. (1998). Motivation and benefits of complex systems approaches in ecology. *Ecosystems*, 1, 449-456.

Monteiro, A.B. & L.D. Faria (2016). The interplay between population stability and food-web topology predicts the occurrence of motifs in complex food-webs. *Journal of Theoretical Biology*, 409, 165-171.

Montoya, J.M., S.L. Pimm & R.V. Sole (2006) Ecological networks and their fragility. *Nature*, 442**,** 259-264.

Moore, H. (1977). The isotopic composition of ammonia, nitrogen dioxide and nitrate in the atmosphere. *Atmospheric Environment (1967)*, 11, 1239-1243.

Moore, J.C., E.L. Berlow, D.C. Coleman, P.C. de Ruiter, Q. Dong, A. Hastings *et al.* (2004). Detritus, trophic dynamics and biodiversity. *Ecology Letters*, 7, 584-600.

Mori, K., N. Bernier, T. Kosaki & J.F. Ponge (2009). Tree influence on soil biological activity: What can be inferred from the optical examination of humus profiles? *European Journal of Soil Biology*, 45, 290-300.

Mueller, U.G., N.M. Gerardo, D.K. Aanen, D.L. Six & T.R. Schultz (2005). The evolution of agriculture in insects. *Annual Review of Ecology Evolution and Systematics*, 36, 563-595.

Mulder, C., A. Boit, S. Mori, J.A. Vonk, S.D. Dyer, L. Faggiano, *et al.* (2012) Distributional (in)congruence of biodiversity-ecosystem functioning. *Advances in Ecological Research,* 46**,** 1-88.

Muller-Landau, H.C. (2007). Predicting the long-terms effects of hunting on plant species composition and diversity in tropical forests. *Biotropica,* 39, 372-384.

Muller-Landau, H.C., S.J. Wright, O. Calderon, R. Condit & S.P. Hubbell (2008). Interspecific variation in primary seed dispersal in a tropical forest. *Journal of Ecology*, 96, 653-667.

N

Nadkarni, N.M. & T.J. Matelson (1992). Biomass and nutrient dynamics of epiphytic litterfall in a neotropical montane forest, Costa Rica. *Biotropica,* 24-30.

Nadkarni, N.M. (1984). Biomass and mineral capital of epiphytes in an *Acer macrophyllum* community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. *Canadian Journal of Botany*, 62, 2223-2228.

Nadkarni, N.M. (1986). The nutritional effects of epiphytes on host trees with special reference to alteration of precipitation chemistry. *Selbyana*, 9, 44-51.

Nair, R.K., M.P. Perks & M. Mencuccini (2017). Decomposition nitrogen is better retained than simulated deposition from mineral amendments in a temperate forest. *Global change biology*, 23, 1711-1724.

Nathan, R. & H.C. Muller-Landau (2000). Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution*, 15, 278-285.

Navarrete-Heredia, J.L. (2001). Beetles associated with *Atta* and *Acromyrmex* ants (Hymenoptera : Formicidae : Attini). *Transactions of the American Entomological Society*, 127, 381-429.

Neubert, M.G. & H. Caswell (2000). Demography and dispersal: Calculation and sensitivity analysis of invasion speed for structured populations. *Ecology*, 81, 1613-1628.

Neutel, A.M., J.A.P. Heesterbeek & P.C. de Ruiter (2002) Stability in real food webs: Weak links in long loops. *Science,* 296**,** 1120-1123.

Nieder, J., J. Prosperí & G. Michaloud, (2001). Epiphytes and their contribution to canopy diversity. In: *Tropical Forest Canopies: Ecology and Management*. Springer, pp. 51-63.

O

Odum, E.P. & H.T. Odum (1953). *Fundamentals of Ecology*. W. B. Saunders Company, Philadelphia.

Okamoto, K.W. (2015). The dynamics of strangling among forest trees. *Journal of Theoretical Biology*, 384, 95-104.

Olff, H., D. Alonso, M.P. Berg, B.K. Eriksson, M. Loreau, T. Piersma, *et al.* (2009) Parallel ecological networks in ecosystems. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 364**,** 1755-1779.

Ollerton, J., R. Winfree & S. Tarrant (2011). How many flowering plants are pollinated by animals? *Oikos,* 120, 321-326.

Osuri, A.M., J. Ratnam, V. Varma, P. Alvarez-Loayza, J.H. Astaiza, M. Bradford *et al.* (2016). Contrasting effects of defaunation on aboveground carbon storage across the global tropics. *Nature Communications*, 7, 11351.

Ott, R.L. & M. Longnecker (2001). *An Introduction to Statistical Methods and Data Analysis.* fifth edn. Duxbury, Pacific Grove, USA.

P

Paine, R.T. (1969) A note on trophic complexity and community stability. *American Naturalist,* 103**,** 91-93.

Parnell, A. & A. Jackson. 2010. *SIAR: stable isotope analysis in R*. R package v. 4.1. 3.

Peguero, G., H.C. Muller-Landau, P.A. Jansen & S.J. Wright (2017). Cascading effects of defaunation on the coexistence of two specialized insect seed predators. *Journal of Animal Ecology*, 86, 136-146.

Peres, C.A. & E. Palacios (2007). Basin-wide effects of game harvest on vertebrate population densities in Amazonian forests: Implications for animal-mediated seed dispersal. *Biotropica*, 39, 304-315.

Peres, C.A., T. Emilio, J. Schietti, S.J.M. Desmouliere & T. Levi (2016). Dispersal limitation induces long-term biomass collapse in overhunted Amazonian forests. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 892-897.

Perez-Mendez, N., P. Jordano, C. Garcia & A. Valido (2016). The signatures of Anthropocene defaunation: cascading effects of the seed dispersal collapse. *Scientific Reports*, 6, 24820.

Peterson, B.J. & B. Fry (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 18, 293-320.

Pilosof, S., M.A. Porter, M. Pascual & S. Kefi (2017) The multilayer nature of ecological networks. *Nature Ecology & Evolution*, 1, 0101.

Pimm, S.L. & T. Brooks (2013). Conservation: Forest fragments, facts, and fallacies. *Current Biology*, 23, R1098-R1101.

Pimm, S.L. (1979). Structure of food webs. *Theoretical Population Biology*, 16, 144-158.

Poveda, C., A. Higuera, P. Urbano & J.D. Ramirez (2017). Ecology of *Trypanosoma cruzi* I genotypes across *Rhodnius prolixus* captured in *Attalea butyracea* palms. *Infection Genetics and Evolution*, 49, 146-150.

Press, M.C. & G.K. Phoenix (2005). Impacts of parasitic plants on natural communities. *New phytologist*, 166, 737-751.

R

Rasband, W.S. (1997). *ImagJ*. U. S. National Institutes of Health, Bethesda, Maryland.

Rather, P.A. & I. Hassan (2014). Human demodex mite: The versatile mite of dermatological importance. *Indian Journal of Dermatology*, 59, 60-66.

Redford, K.H. (1992). The empty forest. *Bioscience*, 42, 412-422.

Reich, P.B. & J. Oleksyn (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences*, 101, 11001-11006.

Reid, A.M. & C.J. Lortie (2012) Cushion plants are foundation species with positive effects extending to higher trophic levels. *Ecosphere*, 3, 96.

Ribbens, E., J.A. Silander & S.W. Pacala (1994). Seedling recruitment in forests - Calibrating models to predict patterns of tree seedling dispersion. *Ecology*, 75, 1794-1806.

Riede, J.O., B.C. Rall, C. Banasek-Richter, S.A. Navarrete, E.A. Wieters, M.C. Emmerson *et al.* (2010). Scaling of food-web properties with diversity and complexity across ecosystems. In: *Advances in Ecological Research* (ed. Woodward, G). Elsevier Academic Press Inc Burlington, pp. 139-170.

Rietkerk, M. & J. Van de Koppel (2008) Regular pattern formation in real ecosystems. *Trends in Ecology & Evolution*, 23**,** 169-175.

Robinson, N.J., E.A. Lazo-Wasem, F.V. Paladino, J.D. Zardus & T. Pinou (2017). Assortative epibiosis of leatherback, olive ridley and green sea turtles in the Eastern Tropical Pacific. *Journal of the Marine Biological Association of the United Kingdom*, 97, 1233-1240.

Rojas-Soto, O.R., I. Macgregor-Fors, C. Diaz-Castelazo, A. Molina-Garcia & C. Maldonado-Hernandez (2014). The role of birds in the acacia-ant interaction: New insights from nest predation. *Ecoscience*, 21, 56-60.

Roscher, C., J. Schumacher, J. Baade, W. Wilcke, G. Gleixner, W.W. Weisser *et al.* (2004). The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, 5, 107-121.

Rosin, C. & J.R. Poulsen (2016). Hunting-induced defaunation drives increased seed predation and decreased seedling establishment of commercially important tree species in an Afrotropical forest. *Forest Ecology and Management*, 382, 206-213.

S

Saint-Beat, B., D. Baird, H. Asmus, R. Asmus, C. Bacher, S.R. Pacella, *et al.* (2015) Trophic networks: How do theories link ecosystem structure and functioning to stability properties? A review. *Ecological Indicators*, 52**,** 458-471.

Sanders, D., C.G. Jones, E. Thébault, T.J. Bouma, T. v. d. Heide, J. v. Belzen, *et al.* (2014) Integrating ecosystem engineering and food webs. *Oikos*, 123**,** 513-524.

Savoca, M.S. & G.A. Nevitt (2014). Evidence that dimethyl sulfide facilitates a tritrophic mutualism between marine primary producers and top predators. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4157-4161.

Sazima, I. & A. Grossman (2006). Turtle riders: remoras on marine turtles in Southwest Atlantic. *Neotropical Ichthyology*, 4, 123-126.

Scarano, F.R. (2002). Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. *Annals of Botany*, 90, 517-524.

Schlesinger, W.H. & P.L. Marks (1977). Mineral cycling and niche of Spanish moss, *Tillandsia usneoides* L. *American Journal of Botany*, 64, 1254-1262.

Scholes, R. & S. Archer (1997). Tree-grass interactions in savannas. *Annual review of Ecology and Systematics*, 28, 517-544.

Schupp, E.W. (1992). The Janzen-Connell model for tropical tree diversity - Population implications and the importance of spatial scale. *American Naturalist*, 140, 526-530.

Silvius, K.M. & J.M.V. Fragoso (2002). Pulp handling by vertebrate seed dispersers increases palm seed predation by bruchid beetles in the northern Amazon. *Journal of Ecology*, 90, 1024-1032.

Silvius, K.M. (2002). Spatio-temporal patterns of palm endocarp use by three Amazonian forest mammals: granivory or 'grubivory'? *Journal of Tropical Ecology*, 18, 707-723.

Sole, R.V. & J.M. Montoya (2001) Complexity and fragility in ecological networks. *Proceedings of the Royal Society B-Biological Sciences*, 268**,** 2039-2045.

St Pierre, J.I. & K.E. Kovalenko (2014). Effect of habitat complexity attributes on species richness. *Ecosphere*, 5, 22.

Stachowicz, J.J. (2001). Mutualism, facilitation, and the structure of ecological communities. *Bioscience*, 51, 235-246.

Stahle, D.W., J.V. Diaz, D.J. Burnette, J.C. Paredes, R.R. Heim, F.K. Fye *et al.* (2011). Major Mesoamerican droughts of the past millennium. *Geophysical Research Letters*, 38, L05703.

Stewart, R.H., J.L. Stewart & W.P. Woodring (1980). Geologic map of the Panama Canal and vicinity, Republic of Panama. In: *Miscellaneous Investigations Series ; map I-1232*. U.S. Geological Survey Reston, U.S.A.

Stone, L. & A. Roberts (1991). Conditions for a species to gain advantage from the presence of competitors. *Ecology*, 72, 1964-1972.

Stoner, K.E., K. Vulinec, S.J. Wright & C.A. Peres (2007). Hunting and plant community dynamics in tropical forests: A synthesis and future directions. *Biotropica*, 39, 385-392.

Stouffer, D.B. & J. Bascompte (2011) Compartmentalization increases food-web persistence. *Proceedings of the National Academy of Sciences of the United States of America*, 108**,** 3648-3652.

Suykerbuyk, W., T.J. Bouma, L.L. Govers, K. Giesen, D.J. de Jong, P. Herman *et al.* (2016). Surviving in changing seascapes: Sediment dynamics as bottleneck for long-term seagrass presence. *Ecosystems*, 19, 296-310.

Svenning, J.C. & F. Skov (2007). Could the tree diversity pattern in Europe be generated by postglacial dispersal limitation? *Ecology Letters*, 10, 453-460.

T

Taniguchi, H., S. Nakano & M. Tokeshi (2003). Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. *Freshwater Biology*, 48, 718-728.

Tanner, E. (1980). Studies on the biomass and productivity in a series of montane rain forests in Jamaica. *The Journal of Ecology*, 68, 573-588.

ter Steege, H., C.A. Nigel, D. Sabatier, C. Baraloto, R.P. Salomao, J.E. Guevara *et al.* (2013). Hyperdominance in the Amazonian tree flora. *Science,* 342, 1243092.

Terborgh, J. & J.A. Estes (2010). Trophic cascades: Predators, prey, and the changing dynamics of nature. Island Press, Washington D.C., USA.

Terborgh, J. (2013). Using Janzen-Connell to predict the consequences of defaunation and other disturbances of tropical forests. *Biological Conservation*, 163, 7-12.

Terborgh, J., G. Nunez-Iturri, N.C.A. Pitman, F.H.C. Valverde, P. Alvarez, V. Swamy *et al.* (2008). Tree recruitment in an empty forest. *Ecology*, 89, 1757-1768.

Tessier, J.T. & D.J. Raynal (2003). Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology*, 40, 523-534.

Thebault, E. & C. Fontaine (2010) Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science,* 329**,** 853-856.

Thompson, R.M., U. Brose, J.A. Dunne, R.O. Hall Jr., S. Hladyz, R.L. Kitching, *et al.* (2012). Food webs: reconciling the structure and function of biodiversity. *Trends in Ecology & Evolution*, 27, 689-697.

Tilman, D. (1981). Tests of resource competition theory using 4 species of Lake-Michigan algae. *Ecology*, 62, 802-815.

Tilman, D. (1986). Nitrogen-limited growth in plants from different successional stages. *Ecology*, 67, 555-563.

Tokeshi, M. & S. Arakaki (2012). Habitat complexity in aquatic systems: fractals and beyond. *Hydrobiologia,* 685, 27-47.

Tozer, W.C., D. Hackell, D. Miers & W. Silvester (2005). Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? *Oecologia*, 144, 628-635.

Turetsky, M.R. (2003). The role of bryophytes in carbon and nitrogen cycling. *The bryologist*, 106, 395-409.

U

Umana, N.H.-N. & W. Wanek (2010). Large canopy exchange fluxes of inorganic and organic nitrogen and preferential retention of nitrogen by epiphytes in a tropical lowland rainforest. *Ecosystems*, 13, 367-381.

V

van den Burg, J. & W. Schaap (1995). Richtlijnen voor mineralentoediening en bekalking als effectgerichte maatregelen in bossen: *Rapport IKC Natuurbeheer* 16 onder redactie van J. van den Burg W. Schaap.

van den Elzen, E., M.A.R. Kox, S.F. Harpenslager, G. Hensgens, C. Fritz, M.S.M. Jetten, *et al.* (2017). Symbiosis revisited: phosphorus and acid buffering stimulate N-2 fixation but not Sphagnum growth. *Biogeosciences*, 14, 1111-1122.

van der Heide, T., L.L. Govers, J. de Fouw, H. Olff, M. van der Geest, M.M. van Katwijk, *et al.* (2012). A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science*, 336, 1432-1434.

van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69-72.

van der Zee, E.M., E. Tielens, S. Holthuijsen, S. Donadi, B.K. Eriksson, H.W. van der Veer, *et al.* (2015) Habitat modification drives benthic trophic diversity in an intertidal soft-bottom ecosystem. *Journal of Experimental Marine Biology and Ecology*, 465**,** 41-48.

van der Zee, E.M., C. Angelini, L.L. Govers, M.J.A. Christianen, A.H. Altieri, K.J. van der Reijden, *et al.* (2016) How habitat-modifying organisms structure the food web of two coastal ecosystems. *Proceedings of the Royal Society B-Biological Sciences*, 283, 20152326.

van Putten, B., M.D. Visser, H.C. Muller-Landau & P.A. Jansen (2012). Distorted-distance models for directional dispersal: A general framework with application to a wind-dispersed tree. *Methods in Ecology and Evolution*, 3, 642-652.

Van Stan, J.T., A. Stubbins, T. Bittar, J.S. Reichard,, K.A. Wright & R.B. Jenkins (2015). *Tillandsia usneoides* (L.) L.(Spanish moss) water storage and leachate characteristics from two maritime oak forest settings. *Ecohydrology*, 8, 988-1004.

Vanthomme, H., B. Belle & P.M. Forget (2010). Bushmeat hunting alters recruitment of large-seeded plant species in Central Africa. *Biotropica*, 42, 672-679.

Verdonschot, R.C.M., K. Didderen & P.F.M. Verdonschot (2012) Importance of habitat structure as a determinant of the taxonomic and functional composition of lentic macroinvertebrate assemblages. *Limnologica*, 42**,** 31-42.

Verweij, M.C., I. Nagelkerken, D. de Graaff, M. Peeters, E.J. Bakker & G. van der Velde (2006). Structure, food and shade attract juvenile coral reef fish to mangrove and seagrass habitats: a field experiment. *Marine Ecology Progress Series*, 306, 257-268.

Visser, M.D. (2016). Trade-offs, enemies & dispersal-Cross-scale comparisons on tropical tree populations. Experimental Plant Ecology. Radboud University Nijmegen.

Visser, M.D., M. Bruijning, S.J. Wright, H.C. Muller-Landau, E. Jongejans, L.S. Comita, *et al.* (2016). Functional traits as predictors of vital rates across the life cycle of tropical trees. *Functional Ecology*, 30, 168-180.

Visser, M.D., H.C. Muller-Landau, S.J. Wright, G. Rutten & P.A. Jansen (2011). Tri-trophic interactions affect density dependence of seed fate in a tropical forest palm. *Ecology Letters*, 14, 1093-1100.

Volterra, V. (1926). Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. *Mem. Acad. Lincei Roma*, 2, 31-113.

W

Waage, J.K. & G.G. Montgomery (1976). *Cryptoses-choloepi* - Coprophagous moth that lives on a sloth. *Science*, 193, 157-158.

Wahl, M. (2008). Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. *Biofouling*, 24, 427-438.

Waycott, M., C.M. Duarte, T.J B. Carruthers, R.J. Orth, W.C. Dennison, S. Olyarnik, *et al.* (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 106**,** 12377-12381.

Whittingham, M.J., P.A. Stephens, R.B. Bradbury & F.P. Freckleton (2006). Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, 75, 1182-1189.

Wilkie, D.S., E.L. Bennett, C.A. Peres & A.A. Cunningham (2011). The empty forest revisited. *Year in Ecology and Conservation Biology*, 1223, 120-128.

Williams, R.J. & N.D. Martinez (2000). Simple rules yield complex food webs. *Nature*, 404, 180-183.

Wotton, D.M. & D. Kelly (2011). Frugivore loss limits recruitment of large-seeded trees. *Proceedings of the Royal Society B-Biological Sciences*, 278, 3345-3354.

Wright, J.T. & P.E. Gribben (2017) Disturbance-mediated facilitation by an intertidal ecosystem engineer. *Ecology*, 98**,** 2425-2436.

Wright, J.T., J.E. Byers, J.L. DeVore & E.E. Sotka (2014) Engineering or food? Mechanisms of facilitation by a habitatforming invasive seaweed. *Ecology*, 95**,** 2699-2706.

Wright, S.J. & H.C. Duber (2001). Poachers and forest fragmentation alter seed dispersal, seed survival and seedling recruitment in the palm *Attalea butyraceae*, with implications for tropical tree diversity. *Biotropica*, 33, 583-595.

Wright, S.J. (1983). The dispersion of eggs by a bruchid beetle among *Scheelea* palm seeds and the effect of distance to the parent palm. *Ecology*, 64, 1016-1021.

Wright, S.J., A. Hernandez & R. Condit (2007a). The bushmeat harvest alters seedling banks by favoring lianas, large seeds, and seeds dispersed by bats, birds, and wind. *Biotropica*, 39, 363-371.

Wright, S.J., K.E. Stoner, N. Beckman, R.T. Corlett, R. Dirzo, H.C. Muller-Landau, *et al.* (2007b). The plight of large animals in tropical forests and the consequences for plant regeneration. *Biotropica*, 39, 289-291.

Wright, S.J., H. Zeballos & I. Dominguez (2000). Poachers alter mammal abundance, seed dispersal, and seed predation in a neotropical forest. *Conservation Biology*, 14, 227-239.

X

Xiao, S., L. Zhao, J.L. Zhang, X.T. Wang & S.Y. Chen (2013). The integration of facilitation into the neutral theory of community assembly. *Ecological Modelling*, 251, 127-134.

Y

Yavitt, J.B. (2000). Nutrient dynamics of soil derived from different parent material on Barro Colorado Island, Panamá. *Biotropica*, 32, 198-207.

Young, O.P., and T.C. Lockley (1989). Spiders of Spanish moss in the delta of Mississippi. *Journal of Arachnology*, 17, 143-148.

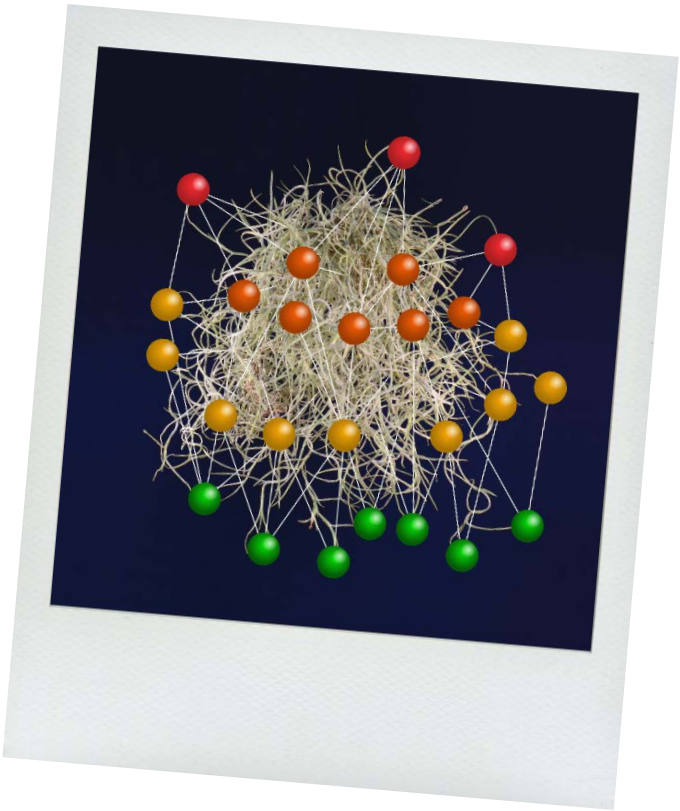
Z

Zhao, Q.H., Y. Pan, J.N. Griffin, J.Z. Sun & S.C. Sun (2016) Contrasting trophic-cascade effects driven by variation in morphology of the perches used by a larval damselfly. *Freshwater Biology*, 61**,** 693-701.

Zhu, Y., L.S. Comita, S.P. Hubbell & K.P. Ma (2015). Conspecific and phylogenetic density-dependent survival differs across life stages in a tropical forest. *Journal of Ecology*, 103, 957-966.

Zhu, Y., S.A. Queenborough, R. Condit, S.P. Hubbell, K.P. Ma & L.S. Comita (2018). Density-dependent survival varies with species life-history strategy in a tropical forest. *Ecology Letters*, 21, 506-515.

Zotz, G. & P. Hietz (2001). The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of experimental botany*, 52, 2067-2078.



8. Summary

Vervang door opgemaakte versie

Every organism in nature is intertwined in a complex web of interactions with other organisms. Together these organisms and their interactions constitute the communities and ecosystems as we know them. By studying individual interactions, or multi-interaction networks, we learn more about the patterns and mechanisms that occur in nature so we can better understand, protect and restore it. Food webs are networks in which all organisms and their food interactions, i.e. trophic interactions, are depicted. Classically, food webs are used to study multiple interactions simultaneously in order to get a better grip on the functioning of the community as a whole. But more and more studies show that interactions outside of the food web, the so-called non-trophic interactions, may also be important for individual species and even for the functioning of entire ecosystems. So to get a grip on the total dynamics within an ecosystem trophic interactions need to be integrated with non-trophic interactions. However, empirical work on this subject remains scarce and mainly theoretical in nature.

Within an ecosystem, not all species play an equally important role. Some have been found to be very important for the functioning of the entire ecosystem. Foundation species are spatially dominant, sessile species that facilitate the local community. These species are often described primarily from the perspective of their non-trophic roles, modifying their habitat and mitigating physical stress for themselves and other species. Therefore, the interplay of trophic and non-trophic interactions in communities structured by foundation species is a very interesting subject to investigate. For this thesis I studied: 1. how foundation species structure food webs, 2. the trophic and non-trophic roles they play and 3. how these foundation species are reciprocally affected by the facilitated species.

First we investigated the effect of foundation species on the food web (Chapter 2). Within the same ecosystem we compared the food webs with a dominant foundation species and "bare" plots, without this foundation species, such as for example branches with Spanish moss festoons and nearby branches without these festoons. Overall, we examined food webs in 7 ecosystems, each with a dominant foundation species: seagrass, mussel beds, cord grass, watermilfoil, water-starwort, marram grass and Spanish moss. The ecosystems in which these species occurred varied from marine to freshwater to terrestrial, such that the results of this study are more generally applicable. This work showed that foundation species-dominated food webs on average held more than twice as many species compared to the ‘bare’ food webs and also the food webs with foundation species were more complex (i.e. more trophic interactions per species). This increase in size and complexity was found in all ecosystems we studied. Also, many other properties of the studied food webs, such as connectance or vulnerability (average number of predators per species), changed accordingly. The increase in species could be the result of the foundation type serving as the most important food source. However, our analyses showed that the foundation species had less trophic interactions than

other species in the food web, suggesting that the facilitating effect of the foundation species on the food web was primarily non-trophic in nature.

The next question we asked was in what way the food webs had increased. What kind of species had been facilitated by the foundation species within these 7 ecosystems? Did the food change in specific ways, (i.e. did the number of trophic levels increase or only more species in a single trophic layer, for example, herbivores), or was the food as a whole growing in a more random manner? We have studied this in a model by 'pruning' the foundation species-dominated food webs. That means we randomly removed species from the food webs, until these pruned food webs were the same size as the food webs in the same system without foundation type (the 'bare plots'). We then calculated the properties of these pruned networks and compared them with the real food webs sampled at the ‘bare spots’. We found that the ‘bare’ and ‘pruned’ food webs were very similar. This suggests a large degree of random expansion of the food web when it is facilitated by a foundation species. However, four food web properties did not match between the modelled food webs and the real bare food webs. The number of ‘basal species’ (mostly plants) was higher in the ‘bare' food webs, while the average trophic level, the average chain length and the number of carnivores was lower than expected from the randomly pruned food webs (the models). This suggests that the species in the foundation species dominated-food webs had on average a higher average trophic level and that relatively more carnivorous species were present. While the basal, often sessile, species, may not have increased proportionally because of spatial competition with the foundation species.

Because the results in Chapter 2 suggest an important non-trophic influence of the foundation species on the community, we experimentally tested the relative effect of the trophic versus non-trophic facilitation on the species community in Chapter 3. Also, we tested how the quantity of foundation species contributes to the facilitation and structure of a community. To this end we conducted an experiment with Spanish moss (as shown on the cover of this thesis). We removed all animals (macroinvertebrates) from Spanish moss in 4 different quantities. We also matched the quantities of the real plants with identical plastic mimic plants. Because the plastic mimic plants do create habitat, but cannot be eaten, we were able to disentangle the trophic and non-trophic effects of Spanish moss in this way. We placed these mimic and real plants in a host tree (the natural habitat of Spanish moss) and added a bare branch as a control. Then we let these branches and plants colonize for three months. We then identified and counted the animals that colonized the bare branched and the real and fake plants. This experiment clearly showed a dual role of Spanish moss strongly facilitating the community in both trophic and non-trophic ways. Furthermore, we found that the proportion of trophic versus non-trophic facilitation by Spanish moss seemed to be independent of the amount of Spanish moss. This study showed that the trophic role of Spanish moss is mainly through the

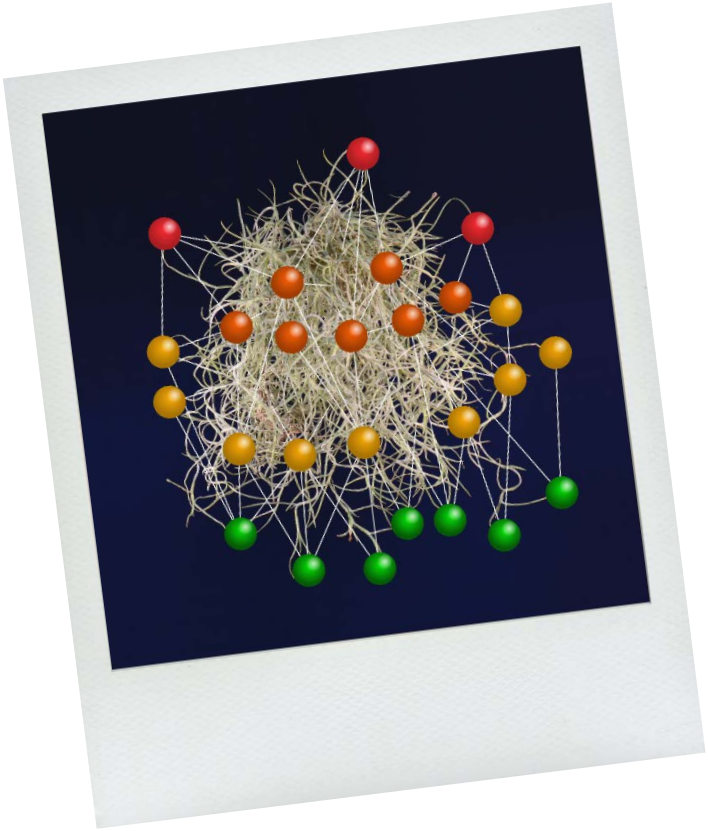
decomposition of dead Spanish moss in the form of detritus, a way that is currently not sufficiently integrated in food web studies.

The important role of Spanish moss in the form of detritus is also apparent from the next study that we carried out (Chapter 4). Spanish moss is a secondary foundation species, the primary foundation species being the tree that is host to the Spanish moss. Epiphytes such as Spanish moss are usually seen as commensalistic (advantage for the epiphyte, but no apparent effect on the host itself) or as parasitic (negative effect on the host). The host trees from the studied location appeared to be limited in their growth by a lack of nitrogen. Spanish moss could potentially influence this, for example by taking away nitrogen from the tree or by making extra nitrogen available for the tree through decomposition. To investigate the role of Spanish moss in the nitrogen budget of the tree, we investigated how Spanish moss obtains its nitrogen. Our research showed that Spanish moss most likely absorbs nitrogen from wet and / or dry deposition or as NH_3 gas, but not from nitrogen fixation, dust or the host tree itself. We also found that Spanish moss absorbed a negligible part of the wet / dry deposition in its biomass. When it dislodges, Spanish moss can be used by the tree as a sort of slow-release fertilizer of organic nitrogen, which is a more easily accessible form of nitrogen. Our research showed that Spanish moss may contribute between 5 and 25% to the nitrogen budget of the host, depending on the amount of Spanish moss. This study indicates the relationship between this primary (the tree) and secondary foundation species (the Spanish moss) as a mutualism, whereby both species have an advantage.

The reciprocity of an interaction with the foundation species has also been explored in a classical seed spreading plant mutuality (Chapter 5). Many plant species depend on mutualistic interactions with pollinators and / or animal seed dispersers for their dispersal. They also often have to deal with, to them, negative interactions with animal species that predate on the seeds. To date it remains unclear what the effect is on individual plant species if these interactions break down by human disturbances, for example by hunting the seed dispersers. In literature it is often suggested that local accumulation of undispersed seeds under the mother tree, via a decrease of the seed dispersers, will lead to a strong increase in the number of seed predators, with a suggested decrease of plant fitness as a result. However, this hypothesis lacks empirical evidence. By means of a simple theoretical model with which we calculated the population growth over increasing plant densities, we have studied this seed disperser-plant interaction. From this model we could then to derive population growth at low plant densities and negative density dependence of this population growth at higher densities. Based on this model, we hypothesized that local plant numbers will increase in the absence of seed dispersers, unless overcompensation occurs from seed predators. We then tested these model findings in the palm *Attalea butyracea* and concluded that the seed predators do not overcompensate for the increase in seeds in this case study. Hunting of the seed dispersers of *Attalea* actually

effectuated a five-fold increase in the plant numbers of this palm. Furthermore, in other studies on other plant-seed disperser relationships we also found indications that this overcompensation of seed predation does not occur when seed disperser numbers decline. Therefore, based on our theoretical model and the empirical data, we expect that animal-dispersed plants will generally locally increase if this interaction is broken down.

In conclusion, I have shown in this thesis that non-trophic interactions play an important role in species communities and the structuring of food webs. Furthermore, I found that the functional role of foundation species through detritus can also play an important role in the facilitation of communities. In addition, I have found that foundation species depend on complex reciprocal interactions with the species they facilitate. This knowledge furthers our understanding the complexity of species interactions and the ecosystems they are part of, allowing us to better protect and restore both individual species and entire ecosystems.



9. Samenvatting

Vervang door opgemaakte versie

Organismen in de natuur zijn altijd verbonden met andere organismen in een complex web van interacties. Samen vormen deze organismen met hun interacties de gemeenschappen en ecosystemen zoals wij die kennen. Door bestudering van individuele interacties, dan wel multi-interactie-netwerken, leren we meer over de patronen en mechanismen die zich voordoen in de natuur. Hierdoor kunnen we de natuur beter begrijpen, beschermen en herstellen. Voedselwebben zijn netwerken waarin alle organismen en hun voedselinteracties, ofwel trofische interacties, worden weergegeven. Voedselwebben zijn een klassieke manier om meerdere interacties tegelijk te bestuderen, om zo meer grip te krijgen op de werking van de gemeenschap als geheel. Maar steeds meer studies laten zien dat interacties die buiten het voedselweb omgaan, de zogenaamde non-trofische interacties, ook van groot belang kunnen zijn voor individuele soorten en zelfs voor het functioneren van een heel ecosysteem. Om dus een goed beeld te krijgen van de dynamieken binnen een soortengemeenschap zullen voedselwebben moeten worden verweven met de non-trofische interacties die parallel aan elkaar plaatsvinden. Hier is echter nog weinig empirisch werk over en blijft daardoor vooral theoretisch van aard.

Binnen een ecosysteem hebben niet alle soorten een even grote rol. Sommige zijn heel belangrijk voor het functioneren van het ecosysteem en anderen minder. Funderingssoorten zijn ruimtelijk dominante, sessiele soorten die de lokale gemeenschap faciliteren. Deze soorten worden vaak vooral beschreven vanuit hun non-trofische rol, zoals habitat modificatie en stress mitigatie. Habitat modificatie is het veranderen van de fysieke omgeving (door bijvoorbeeld meer structuur aan te brengen en stress mitigatie houdt in dat het omgevingsstress verlaagd voor organismen. Funderingsoorten zijn dus bij uitstek geschikt voor onderzoek naar het samenspel van trofische en non-trofische interactie. In deze soortengemeenschappen gedomineerd door funderingssoorten heb ik onderzoek gedaan naar: 1. op welke manier funderingssoorten het voedselweb structureren, 2. naar de trofische en non-trofische rol die ze spelen en 3. naar op welke manier deze funderingssoorten trofisch of non-trofisch worden beïnvloed door de via henzelf gefaciliteerde soorten.

Eerst hebben we onderzocht welk effect funderingssoorten hebben op het voedselweb (Hoofdstuk 2). Dit hebben we gedaan door voedselwebben met een dominante funderingssoort te vergelijken met ‘kale’ plekken binnen hetzelfde ecosysteem zonder deze funderingssoort, zoals bijvoorbeeld takken met Spaans mos en dichtbijzijnde takken zonder Spaans mos. We hebben de voedselwebben in 7 ecosysteemtypen met funderingssoorten onderzocht; namelijk ecosystemen met: zeegras, mosselbedden, slijkgras, teer vederkruid, sterrekroos, helmgras en Spaans mos als funderingssoort. De ecosystemen waarin deze soorten voorkomen varieerden van marine tot zoetwater en van aquatisch tot terrestrisch, waardoor de uitkomsten van dit onderzoek meer generiek toepasbaar zijn. Uit dit onderzoek bleek dat funderingssoorten het aanwezige voedselweb gemiddeld meer dan 2 keer zo veel soorten bevatten ten opzichte van

de plekken zonder funderingssoort en ook werd het voedselweb complexer (i.e. meer trofische interacties per soort). In alle ecosystemen die we onderzochten troffen we een toename van soorten en voedselwebcomplexiteit aan. Ook bleek dat veel andere eigenschappen van de voedselwebben, zoals de relatieve gelinktheid (connectance) of het gemiddelde aantal predatoren van soorten (vulnerability), vergelijkbaar veranderden binnen de onderzochte ecosystemen. De toename van soorten zou mogelijk verklaard kunnen worden doordat de funderingssoort als belangrijkste voedselbron dient. Uit onze analyses bleek echter dat de funderingsoort gemiddeld minder trofische interacties had dan andere soorten in het voedselweb. Dit suggereert dat de faciliterende werking van de funderingssoorten op het voedselweb voornamelijk op non-trofische manieren tot stand komt.

De volgende vraag die we stelden was op welke manier de voedselwebben waren gegroeid, dus wat voor soorten waren gefaciliteerd door de funderingssoorten in deze 7 ecosystemen. Was het voedselweb op een specifieke manier veranderd, (i.e. waren er bijvoorbeeld meer trofische lagen bij gekomen of alleen meer soorten van één trofische laag, bijvoorbeeld de herbivoren), of was het voedselweb in zijn geheel gegroeid op een meer willekeurige manier? Dit hebben we modelmatig onderzocht door de voedselwebben die gefaciliteerd werden door een funderingssoort te 'snoeien'. Dat betekent dat we willekeurig soorten lieten verdwijnen, totdat deze gesnoeide voedselwebben even groot waren als de voedselwebben in hetzelfde systeem zonder funderingssoort (de 'kale plekken'). Door de eigenschappen van deze gemodelleerde gesnoeide voedselwebben te berekenen en te vergelijken met de echte voedselwebben van de 'kale plekken', kwamen wij erachter dat de echte en gemodelleerde voedselwebben veel op elkaar leken. Dit duidt dus op een grote mate van willekeurige uitbreiding van het voedselweb wanneer het gefaciliteerd wordt door een funderingssoort. Echter kwamen vier voedselwebeigenschappen niet overeen tussen de gemodelleerde gesnoeide voedselwebben en de echte 'kale plekken'-voedselwebben. Het aantal 'basale soorten' helemaal onderaan de voedselketen (meestal planten) was hoger in de echte 'kale plekken'-voedselwebben, terwijl het gemiddelde trofische niveau, de gemiddelde ketenlengte en het aantal carnivoren (vleeseters) lager waren dan werd verwacht op basis van de willekeurig gesnoeide voedselwebben (de modellen). Dit duidt erop dat de soorten in voedselwebben met funderingssoort gemiddeld hogere trofische niveaus hebben en dat er meer carnivore soorten aanwezig zijn. Terwijl de basale soorten, vaak sessiele (niet bewegende) soorten, wellicht niet evenredig toenemen door ruimtelijke competitie met de funderingssoorten zelf.

Omdat we in Hoofdstuk 2 vonden dat funderingssoorten een grote non-trofische invloed op de gemeenschap leek te hebben, hebben wij in hoofdstuk 3 experimenteel getest welk aandeel trofische dan wel non-trofische interacties hadden voor het faciliteren van de soortgemeenschap, en daarbij hoe de hoeveelheid van een funderingssoort bijdraagt de facilitatie en structuur van een soortgemeenschap. Dit hebben we getest door middel van een

experiment met Spaans mos (te zien op de omslag van dit proefschrift). We hebben Spaans mos in 4 verschillende hoeveelheden, en ook kale takken, vrijgemaakt van dieren (m.n. macroinvertebraten). Ook hebben wij de echte planten met vrijwel identieke plastic namaakplanten vergeleken. Omdat de plastic planten wel habitat creëren, maar niet gegeten kunnen worden, konden we op deze manier de trofische en non-trofische effecten van Spaans mos uit elkaar halen. Dit alles hebben we in een gastheerboom gehangen (de natuurlijke groeiplaats van Spaans mos) en hebben we 3 maanden laten hangen en daarna de dieren geïdentificeerd en geteld die de kale takken, de echte en de namaakplanten hadden gekoloniseerd. Uit dit experiment bleek duidelijk dat Spaans mos zowel op een trofische als non-trofisch manier de soortgemeenschap sterk bevorderde. Verder vonden we dat het aandeel trofische dan wel non-trofische facilitatie waarmee Spaans mos de soortgemeenschap stimuleerden onafhankelijk leek te zijn van de hoeveelheid Spaans mos. Uit deze studie bleek verder dat het trofische aandeel van Spaans mos vooral werd veroorzaakt door de afbraak van dood Spaans mos in de vorm van detritus, een route die in voedselwebstudies vaak onderbelicht blijft.

Dat Spaans mos een belangrijke rol heeft in de vorm van detritus blijkt ook uit de volgende studie (hoofdstuk 4) die we hebben uitgevoerd. Spaans mos is namelijk een secundaire funderingssoort, de primaire funderingssoort is de boom die gastheer is van Spaans mos. Epifyten zoals Spaans mos, worden meestal gezien als commensalistisch (heeft voordeel van de gastheer, maar heeft zelf geen effect op de gastheer) of als parasitair (negatief voor de gastheer). De gastheerbomen bleken op de onderzochte locatie in hun groei geremd te zijn door een gebrek aan stikstof. Potentieel zou Spaans mos hier invloed op uit kunnen oefenen, bijvoorbeeld door het wegnemen van stikstof of juist door het beschikbaar maken van extra stikstof voor de boom via detritus. Om te onderzoeken welke rol Spaans mos in de stikstofhuishouding van de boom speelde hebben we onderzocht hoe Spaans mos aan zijn stikstof komt. Uit ons onderzoek bleek dat Spaans mos waarschijnlijk zijn stikstof uit natte en/of droge depositie of als NH_3-gas opneemt, en niet uit stikstof-fixatie of uit de gastheerboom zelf. We vonden verder dat Spaans mos een verwaarloosbaar deel van de natte/droge depositie opneemt in haar biomassa. Nadat het op de grond valt kan Spaans mos als detritus als een soort langzame-afgifte bemester van goed opneembaar, organisch stikstof fungeren voor de boom. Uit ons onderzoek bleek dat Spaans mos een bijdrage kan leveren van tussen de 5 en 25% op het stikstofbudget van de gastheer, afhankelijk van de hoeveelheid Spaans mos. Hiermee kunnen we de relatie tussen deze primaire (de boom) en secundaire funderingssoort (het Spaans mos) aanduiden als een mutualisme, waarbij beide soorten dus een voordeel hebben.

De wederkerigheid van een interactie met de funderingssoort hebben we verder uitgezocht in een klassiek zaadverpreider-plantmutualisme (Hoofdstuk 5). Veel plantensoorten zijn voor

hun verspreiding afhankelijk van mutualistische interacties met bestuivers en/of zaadverspreiders. Ook hebben ze vaak te maken met, voor hun, negatieve interacties met diersoorten welke de zaden eten. Het is tot heden onbekend wat voor effect het op individuele plantensoorten heeft als deze interacties doorbroken worden door menselijk handelen, bijvoorbeeld door het jacht op de zaadverspreiders. In de literatuur wordt vaak gesuggereerd dat de lokale ophoping van onverspreide zaden onder de moederboom door een afname van de zaadverspreiders (bijvoorbeeld door jacht op deze soorten) zal leiden tot een nog sterkere toename van de zaadpredatoren, met een afname de fitness van de plant tot gevolg. Deze hypothese is echter weinig empirisch bewijs. Door middel van een simpel theoretisch model waarbij de populatiegroei over plantdichtheden worden berekend hebben we deze zaadverspreider-plant interactie bestudeerd. Uit dit model konden we vervolgens de populatiegroei bij lage plantdichtheden en negatieve dichtheidsafhankelijkheid van deze populatiegroei bij hogere dichtheden afleiden Op basis van dit model bleek dat lokale plantaantallen zullen toenemen bij afwezigheid van zaadverspreiders, tenzij er overcompensatie optreedt vanuit zaadpredatoren. Vervolgens hebben we deze modelbevindingen getest in de palm *Attalea butyracea* en kwamen tot de conclusie dat de zaadpredatoren niet overcompenseren voor de toename in zaden in deze casus. Daardoor kan jacht op de zaadpredatoren van *Attalea* zorgen voor een vervijfvoudiging van de plantenaantallen van deze palm. Verder vonden we in de literatuur ook aanwijzingen dat deze overcompensatie in andere plant-zaadverspreider relaties ook niet plaatsvindt bij afname van de verspreider. Daarom verwachten we op basis van ons theoretische model en de gevonden empirische data dat dierverspreide planten lokaal zullen toenemen in het geval deze interactie wordt doorbroken.

Concluderend heb ik in dit proefschrift laten zien dat ook non-trofische interacties een belangrijke rol spelen voor soortsgemeenschappen en de vorming van voedselwebben. Daarnaast vond ik dat de trofische rol van funderingsoorten via detritus ook een belangrijke rol kan spelen in de facilitatie van gemeenschappen. Daarbij heb ik gevonden dat ook funderingsoorten afhankelijk zijn van complexe wederzijdse interacties met gefaciliteerde soorten. Met deze kennis kunnen we de complexiteit van deze ecosystemen en soortinteracties steeds beter te begrijpen en zodat we nu en in de toekomst zowel individuele soorten als hele ecosystemen beter kunnen beschermen en herstellen.


Kornuit

10. Dankwoord

Vervang door opgemaakte versie

Nu mijn promotie toch echt voltooid is, is het tijd om terug te kijken. Er waren veel momenten waarop ik sterk heb getwijfeld of dit boekje hier ooit zou liggen. Ik moest van ver komen, maar het ligt er nu toch. Dit kleine wondertje heb ik mede te danken aan een aantal mensen die ik heb ontmoet tijdens mijn tijd bij de ecologie-afdeling van de Radboud Universiteit. Ik heb mij in wing1.1 vanaf dag één thuis en gewaardeerd gevoeld. Eerst tijdens mijn masterstages en later als promovendus. Hier heb ik leren vragen stellen, eerlijk naar mijzelf kijken en ben ik mezelf geworden. Veel mensen hebben aan die ontwikkeling bijgedragen, die ik op deze plek zou willen bedanken.

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11. CV

Vervang door opgemaakte versie

I, Annieke (A.C.W.) Borst, was born on the 20th of June 1987 in Zwolle. I grew up in the tiny city of Hattem, Gelderland, the Netherlands. After graduating from the Greijdanus College in Zwolle in 2005, I did my Bachelors in Maastricht, the Netherlands, where I studied Biomedical Sciences. In 2010 I started my pre-master in Biology at the Radboud University Nijmegen, the Netherlands. In 2013 I received my Masters there on 1) the biogeochemistry of carp bioturbation and 2) the density dependent population dynamics of *Attalea butyracea*. During this period I already started my PhD-position at Aquatic Ecology & Environmental Biology. As you can read in this thesis, I studied ways to disentangle trophic and non-trophic interactions, focusing on foundation species, which are suggested to facilitate communities mostly non-tropically. I am now going to expand on and apply this knowledge in my new job as a researcher at Wageningen Environmental Research, further exploring the mechanisms underlying biodiversity in freshwater systems.

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Scientific Publications

Borst A.C.W., W.C.E.P. Verberk, W.C.E.P., C. Angelini, J. Schotanus, J.W. Wolters, M.J.A. Christianen, E. van der Zee, M. Derksen-Hooijberg, T. van der Heide (2018). Foundation species enhance food web complexity through non-trophic facilitation. *PLoS One*, 13, 1-15.

Christianen, M.J.A., T. van der Heide, S J. Holthuijsen, K.J. van der Reijden, **A.C.W. Borst** & H. Olff (2017). Biodiversity and food web indicators of community recovery in intertidal shellfish reefs. *Biological Conservation*, 213**,** 317-324.

Derksen-Hooijberg M., C. Angelini, L.P. Lamers, **A. Borst**, A. Smolders, J.R. Hoogveld, H. Paoli, J. de Koppel, B.R. Silliman, T. van der Heide (2017). Mutualistic interactions amplify salt marsh restoration success. *J Appl Ecol*, 55, 405–14.

Derksen-Hooijberg, M., T. van der Heide, L.P.M. Lamers, **A. Borst,** A.J.P. Smolders, L.L. Govers, J.R.H. Hoogveld & C. Angelini (2018). Burrowing crabs weaken mutualism between foundation species. *Ecosystems*, 1–14.

Derksen-Hooiberg M., J. Hoogveld, C. Angelini, L.P.M. Lamers, **A. Borst**, A. Smolders, S. Harpenslager, L.L. Govers, T. van der Heide (2019). Repetitive drought weakens a salt marsh mutualism. *J. Ecology*. *Accepted*

Other Publications

Veldhuis, M., **A. Borst**, M. Scheepens, Ineke Barten, A. Dees, M. Moeleker, B. Brugmans, R. Verdonschot (2019). De ecologische meerwaarde van het aanbrengen van grindbedden in de Tongelreep. *H2O. Accepted*

Radboud Talks pitch: youtu.be/MgofWL84ats

Notes